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(54) **Biosynthetic osteogenic proteins and osteogenic devices containing them**

Biosynthetische knochenbildende Proteine, und sie enthaltende knochenbildende Vorrichtungen

Protéines biosynthétiques ostéogènes, et dispositifs contenant les-dites

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(73) Proprietor: **STRYKER CORPORATION**
Kalamazoo, Michigan 49003-4085 (US)

(72) Inventors:
- **Opperman, Hermann**
Medway, Massachusetts 02053 (US)
- **Kuberasampath, Thangavel**
Medway, Massachusetts 02053 (US)
- **Rueger, David C.**
West Roxbury, Massachusetts 02132 (US)

(74) Representative: **Price, Vincent Andrew et al**
Fry Heath & Spence LLP
The Gables
Massetts Road
Horley Surrey RH6 7DQ (GB)

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Description

[0001] This invention relates to osteogenic devices, to synthetic genes encoding proteins which can induce osteogenesis in mammals and methods for their production using recombinant DNA techniques, to synthetic forms of osteogenic protein, and to bone and cartilage repair procedures using osteogenic device comprising the synthetic proteins.

[0002] Mammalian bone tissue is known to contain one or more proteinaceous materials, presumably active during growth and natural bone healing, which can induce a developmental cascade of cellular events resulting in endochondral bone formation. This active factor (or factors) has variously been referred to in the literature as bone morphogenetic or morphogenic protein, bone inductive protein, osteogenic protein, osteogenin, or osteoinductive protein.

[0003] The developmental cascade of bone differentiation consists of recruitment of mesenchymal cells, proliferation of progenitor cells, calcification of cartilage, vascular invasion, bone formation, remodeling, and finally marrow differentiation (Reddi (1981) Collagen Rel. Res. 1:209-226).

[0004] Though the precise mechanisms underlying these phenotypic transformations are unclear, it has been shown that the natural endochondral bone differentiation activity of bone matrix can be dissociatively extracted and reconstituted with inactive residual collagenous matrix to restore full bone induction activity (Sampath and Reddi, (1981) Proc. Natl. Acad. Sci. USA 78:7599-7603). This provides an experimental method for assaying protein extracts for their ability to induce endochondral bone in vivo.

[0005] This putative bone inductive protein has been shown to have a molecular mass of less than 50 kilodaltons (kD). Several species of mammals produce closely related protein as demonstrated by cross species implant experiments (Sampath and Reddi (1983) Proc. Natl. Acad. Sci. USA 80:6591-6595).

[0006] The potential utility of these proteins has been widely recognized. It is contemplated that the availability of the pure protein would revolutionize orthopedic medicine, certain types of plastic surgery, and various periodontal and craniofacial reconstructive procedures.

[0007] The observed properties of these protein fractions have induced an intense research effort in various laboratories directed to isolating and identifying the pure factor or factors responsible for osteogenic activity. The current state of the art of purification of osteogenic protein from mammalian bone is disclosed by Sampath et al. (Proc. Natl. Acad. Sci. USA (1987) 80). Urist et al. (Proc. Soc. Exp. Biol. Med. (1984) 173:194-199) disclose a human osteogenic protein fraction which was extracted from demineralized cortical bone by means of a calcium chloride-urea inorganic-organic solvent mixture, and retrieved by differential precipitation in guanidine-hydrochloride and preparative gel electrophoresis. The authors report that the protein fraction has an amino acid composition of an acidic polypeptide and a molecular weight in a range of 17-18 kD.

[0008] Urist et al. (Proc. Natl. Acad. Sci. USA (1984) 81:371-375) disclose a bovine bone morphogenetic protein extract having the properties of an acidic polypeptide and a molecular weight of approximately 18 kD. The authors reported that the protein was present in a fraction separated by hydroxyapatite chromatography, and that it induced bone formation in mouse hindquarter muscle and bone regeneration in trephine defects in rat and dog skulls. Their method of obtaining the extract from bone results in ill-defined and impure preparations.

[0009] European Patent Application Serial No. 148,155, published October 7, 1985, purports to disclose osteogenic proteins derived from bovine, porcine, and human origin. One of the proteins, designated by the inventors as a P3 protein having a molecular weight of 22-24 kD, is said to have been purified to an essentially homogeneous state. This material is reported to induce bone formation when implanted into animals.

[0010] International Application No. PCT/087/01537, published January 14, 1988, discloses an impure fraction from bovine bone which has bone induction qualities. The named applicants also disclose putative bone inductive factors produced by recombinant DNA techniques. Four DNA sequences were retrieved from human or bovine genomic or cDNA libraries and apparently expressed in recombinant host cells. While the applicants stated that the expressed proteins may be bone morphogenic proteins, bone induction was not demonstrated. See also Urist et al., EP 0,212,474 entitled Bone Morphogenic Agents.

[0011] Wang et al. (Proc. Nat. Acad. Sci. USA (1988) 85: 9484-9488) discloses the purification of a bovine bone morphogenetic protein from guanidine extracts of demineralized bone having cartilage and bone formation activity as a basic protein corresponding to a molecular weight of 30 kD determined from gel elution. Purification of the protein yielded proteins of 30, 18 and 16 kD which, upon separation, were inactive. In view of this result, the authors acknowledged that the exact identity of the active material had not been determined.

[0012] Wozney et al. (Science (1988) 242: 1528-1534) discloses the isolation of full-length cDNA's encoding the human equivalents of three polypeptides originally purified from bovine bone. The authors report that each of the three recombinantly expressed human proteins are independently or in combination capable of inducing cartilage formation. No evidence of bone formation is reported.

[0013] It is an object of this invention to provide osteogenic devices comprising matrices containing dispersed osteogenic protein capable of bone induction in allogenic and xenogenic implants. Another object is to provide synthetic osteogenic proteins capable of inducing endochondral bone formation in mammals, including humans. Yet another

object is to provide genes encoding non-native osteogenic proteins and methods for their production using recombinant DNA techniques. Another object is to provide novel biosynthetic forms of osteogenic proteins and a structural design for novel, functional osteogenic proteins. Another object is to provide methods for inducing cartilage formation.

[0014] These and other objects and features of the invention will be apparent from the description, drawings, and claims which follow.

Summary of the Invention

[0015] This invention involves osteogenic devices which, when implanted in a mammalian body, can induce at the locus of the implant the full developmental cascade of endochondral bone formation and bone marrow differentiation. Suitably modified as disclosed herein, the devices also may be used to induce cartilage formation. The devices comprise a carrier material, referred to herein as a matrix, having the characteristics disclosed below, containing dispersed osteogenic protein in the form of a biosynthetic construct.

[0016] Key to these developments was the successful preparation of substantially pure osteogenic protein by purification from bone, the elucidation of amino acid sequence and structure data of the native osteogenic protein, and insights'involving study of the DNA and amino acid sequences of the natural source product. A protocol was developed which results in retrieval of active, substantially pure osteogenic protein from mammalian bone. Investigation of the properties and structure of the native form osteogenic protein then permitted the inventors to develop a rational design for non-native forms, i.e., forms never before known in nature, capable of inducing bone formation. As far as applicants are aware, the constructs disclosed herein constitute the first instance of the design of a functional, active protein without preexisting knowledge of the active region of a native form nucleotide or amino acid sequence.

[0017] A series of consensus DNA sequences were designed with the goal of producing an active osteogenic protein. The sequences were based on partial amino acid sequence data obtained from the naturally sourced product and on observed homologies with unrelated genes reported in the literature, or the sequences they encode, having a presumed or demonstrated developmental function. Several of the biosynthetic consensus sequences have been expressed as fusion proteins in procaryotes, purified, cleaved, refolded, combined with a matrix, implanted in an established animal model, and shown to have endochondral bone-inducing activity. The currently preferred active proteins comprise sequences designated COPS, COP7, COP16, and OP1. The amino acid sequences of these proteins are set forth below.

```
              1         10                  20            30          40
COP5                 LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPFPLAD
                              50                   60                  70
              HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
                   80              90             100
              ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

```
              1         10                  20            30          40
COP7                 LYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD
                              50                   60                  70
              HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
                   80              90             100
              ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

```
                                                          -10
                                                    PKHHSQRARKKNKN
             1          10           20           30          40
COP16        CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPFPLAD
                         50           60          70
             HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
                 80         90          100
             ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

```
                                                              -5
                                                            HQRQA
             1          10           20           30           40
OP1          CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
                          50           60          70
             YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
                 80         90          100
             ISVLYFDDSSNVILKKYRNMVVRACGCH
```

[0018] In these sequences and all other amino acid sequences disclosed herein, the dashes (-) are used as fillers only to line up comparable sequences in related proteins, and have no other function. Thus, amino acids 45-50 of COP7, for example, are NHAVV. Also, the numbering of amino acids is selected solely for purposes of facilitating comparisons between sequences. Thus, for example, the DF residues numbered at 9 and 10 of COPS and COP7 may comprise residues, e.g., 35 and 36, of an osteogenic protein embodying the invention. Various leader or trailer sequences may be attached to the operative active region provided the osteogenic or chondrogenic activity of the protein is not destroyed.

[0019] Thus, in one aspect, the invention comprises a protein comprising an amino acid sequence sufficiently duplicative of the sequence of COPS, COP7, COP16, or OP1 such that it is capable of inducing endochondral bone formation when properly folded and implanted in a mammal in association with a matrix. Some of these sequences induce cartilage, but not bone. Also, the bone forming materials may be used to produce cartilage if implanted in an avascular locus, or if an inhibitor to full bone development is implanted together with the active protein. Thus, in another aspect, the invention comprises a protein less than about 200 amino acids long (for each chain) including a sequence sufficiently duplicative of the sequence of COPS, COP7, COP16, or OP1 such that it is capable at least of cartilage formation when properly folded and implanted in a mammal in association with a matrix. The phrase "sufficiently duplicative", as used herein, is used to describe proteins having a degree of homology with the specific sequences disclosed herein and other, different amino acids but which nevertheless exhibit osteogenic or chondrogenic activity.

[0020] In one preferred aspect, these proteins comprise species of the generic amino acid sequences:

```
  1          10          20          30          40          50
      LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX
        60         70          80          90         100
QXXVXXXNXXXXPXXCCXPXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX
```

or

```
 1         10          20          30          40          50
CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX
          60          70          80          90         100
QXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX
```

where the letters indicate the amino acid residues of standard single letter code, and the Xs each represent any one of the 22 naturally occurring amino acid residues. Preferred amino acid sequences within the foregoing generic sequences are:

```
 1         10          20          30          40          50
    LYVDFRDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNSTNHAIV
     K S S L  QE VIS E FD Y  E A AY MPESMKAS   VI
     F E K I  DN     L    N  S   Q  ITK F P    TL
         A     S     K
          60          70          80          90         100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
 SI HAI SEQV EP  A  EQMNSLAI FFNDQDK I RK EE T DA H H
    RF    T   S     K DPV V  Y N S    H RN   RS
     N    S                    K        P     E
```

and

```
 1         10          20          30          40          50
CKRHPLYVDFRDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNSTNHAIV
 RRRS K S S L  QE VIS E FD Y  E A AY MPESMKAS   VI
   KE F E K I  DN     L    N  S   Q  ITK F P    TL
    Q     A     S     K
          60          70          80          90         100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
 SI HAI SEQV EP  A  EQMNSLAI FFNDQDK I RK EE T DA H H
    RF    T   S     K DPV V  Y N S    H RN   RS
     N    S                    K        P     E
```

wherein each of the amino acids arranged vertically at each position in the sequence may be used alternatively in various combinations. Note that these generic sequences have 6 and preferably 7 cysteine residues where inter- or intramolecular disulfide bonds can form, and contain other critical amino acids which influence the tertiary structure of the proteins. These generic structural features are found in previously published sequences, none of which have been described as capable of osteogenic activity, and most of which never have been linked with such activity.

[0021] Particular useful sequences include:

```
          1         10                  20                  30                  40
Vgl       CKKRHLYVEFK-DVGWQNWVIAPQGYMANYCYGECPYPLTE
                        50                  60                  70
          ILNGSN--H-AILQTLVHSIEPED-IPLPCCVPTKMSP
              80                  90                  100
          ISMLFYDNNDNVVLRHYENMAVDECGCR

          1         10                  20                  30                  40
DPP       CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD
                        50                  60                  70
          HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS
              80                  90                  100
          VAMLYLNDQSTVVLKNYQEMTVVGCGCR

          1         10                  20                  30                  40
CBMP-2a   CKRHPLYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD
                        50                  60                  70
          HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTELSA
              80                  90                  100
          ISMLYLDENEKVVLKNYQDMVVEGCGCR

          1         10                  20                  30                  40
CBMP-2b   CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPFPLAD
                        50                  60                  70
          HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA
              80                  90                  100
          ISMLYLDEYDKVVLKNYQEMVVEGCGCR

          1         10                  20                  30                  40
CBMP-3    CARRYLKVDFA-DIGWSEWIISPKSFDAYYCSGACQFPMPK
                        50                  60                  70
          SLKPSN--H-ATIQSIVRAVGVVPGIPEPCCVPEKMSS
              80                  90                  100
          LSILFFDENKNVVLKVYPNMTVESCACR

          1         10                  20                  30                  40
COP1           LYVDFQRDVGWDDWIIAPVDFDAYYCSGACQFPSAD
                        50                  60                  70
          HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
              80                  90                  100
          ISMLYLDENSTVVLKNYQEMTVVGCGCR
```

```
        1       10         20          30        40
COP3            LYVDFQRDVGWDDWIVAPPGYQAFYCSGACQFPSAD
                         50         60         70
        HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
             80          90         100
        ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

```
        1       10         20          30        40
COP4            LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD
                         50         60         70
        HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
             80          90         100
        ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

Vgl is a known Xenopus sequence heretofore not associated with bone formation. DPP is an amino acid sequence encoded by a Drosophila gene responsible for development of the dorsoventral pattern. OP1 is a region of a natural sequence encoded by exons of a genomic DNA sequence retrieved by applicants. The CBMPs are amino acid sequences comprising subparts of mammalian proteins encoded by genomic DNAs and cDNAs retrieved by applicants. The COPs are totally biosynthetic protein sequences expressed by novel consensus gene constructs, designed using the criteria set forth herein, and not yet found in nature.

[0022] These proteins are believed to be dimers. They appear not to be active when reduced. Various combinations of species of the proteins may exist as heterodimers or homodimers. As far as applicants are aware, the COP5, COP7, COP16, and OP1 constructs constitute the first instances of the design of a bioactive protein without preexisting knowledge of the active region of a native form nucleotide or amino acid sequence.

[0023] The invention thus provides synthetic osteogenic protein produced using recombinant DNA techniques. The protein may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated or mutated forms of native protein, no matter how derived. In view of this disclosure, skilled genetic engineers can design and synthesize genes which encode appropriate amino acid sequences, and then can express them in various types of host cells, including both procaryotes and eucaryotes, to produce large quantities of active synthetic proteins comprising truncated analogs, muteins, fusion proteins, and other constructs mimicking the biological activity of the native forms and capable of inducing bone formation in mammals including humans.

[0024] The synthetic proteins are useful in clinical applications in conjunction with a suitable delivery or support system (matrix). The matrix is made up of particles or porous materials. The pores must be of a dimension to permit progenitor cell migration and subsequent differentiation and proliferation. The particle size should be within the range of 70 - 850 μm, preferably 70 - 420 μm. It may be fabricated by close packing particulate material into a shape spanning the bone defect, or by otherwise structuring as desired a material that is biocompatible (non-inflammatory) and, biodegradable in vivo to serve as a "temporary scaffold" and substratum for recruitment of migratory progenitor cells, and as a base for their subsequent anchoring and proliferation. Currently preferred carriers include particulate, demineralized, guanidine extracted, species-specific (allogenic) bone, and particulate, deglycosglated (or HF treated), protein extracted, demineralized, xenogenic bone. Optionally, such xenogenic bone powder matrices also may be treated with proteases such as trypsin. Other useful matrix materials comprise collagen, homopolymers and copolymers of glycolic acid and lactic acid, hydroxyapatite, tricalcium phosphate and other calcium phosphates.

[0025] The osteogenic proteins and implantable osteogenic devices enabled and disclosed herein will permit the physician to obtain optimal predictable bone formation to correct, for example, acquired and congenital craniofacial

and other skeletal or dental anomalies (Glowacki et al. (1981) Lancet 1:959-963). The devices may be used to induce local endochondral bone formation in non-union fractures, and in other clinical applications including periodontal applications where bone formation is required. Another potential clinical application is in cartilage repair, for example, in the treatment of osteoarthritis.

Brief Description of the Drawing

[0026] The foregoing and other objects of this invention, the various features thereof, as well as the invention itself, may be more fully understood from the following description, when read together with the accompanying drawings, in which:

FIGURE 1 is a comparison of the amino acid sequence of various osteogenic proteins to those of the TGF-beta family. COP1, COP3, COP4, COPS, and COP7 are a family of analogs of synthetic osteogenic proteins developed from the consensus gene that was joined to a leader protein via a hinge region having the sequence D-P-N-G that permitted chemical cleavage at the D-P site (by acid) or N-G (by hydroxylamine) resulting in the release of the analog protein; VGI is a Xenopus protein, DPP is a Drosophila protein; OP1 is a native osteogenic protein; CBMP2a and 2b, and CBMP3 are subparts of proteins disclosed in PCT application 087/01537; MIS is Mullerian inhibitory substance; and "consensus choices" represent various substitutions of amino acids that may be made at various positions in osteogenic proteins;

FIGURE 2A is an E. coli expression vector containing a gene of an osteogenic protein fused to a leader protein;

FIGURE 2B is the DNA sequence comprising a modified trp-LE leader, two Fb domains of protein A, an ASP-PRO cleavage site, and the COPS sequence;

FIGURES 3A and 3B are photomicrographs of implants showing the histology (day 12) of COPS active recombinant protein. A is control (rat matrix alone, 25 mg). B is rat matrix plus COP5, showing +++ cartilage formation and ++ bone formation (see key infra). Similar results are achieved with COP7; and

FIGURE 4 is a schematic representation of the DNA sequence and corresponding amino acid sequence of a consensus gene for osteogenic protein (COPO).

Description

[0027] Purification protocols have been developed which enable isolation of the osteogenic protein present in crude protein extracts from mammalian bone. The isolation procedure enables the production of significant quantities of substantially pure osteogenic protein from any mammalian species, provided sufficient amounts of fresh bone from the species is available. The empirical development of the procedure, coupled with the availability of fresh calf bone, enabled isolation of substantially pure bovine osteogenic protein (BOP). BOP has been characterized significantly; its ability to induce cartilage and ultimately endochondral bone growth in cat, rabbit, and rat have been studied; it has been shown to be able to induce the full developmental cascade of bone formation previously ascribed to unknown protein or proteins in heterogeneous bone extracts; and it may be used to induce formation of endochondral bone in orthopedic defects including non-union fractures. In its native form it is a glycosylated, dimeric protein. However, it is active in deglycosylated form. It has been partially sequenced.

[0028] Elucidation of the amino acid sequence of BOP enabled the construction of a consensus nucleic acid sequence designed as disclosed herein based on the sequence data, inferred codons *for* the sequences, and observation of partial homology with known genes.

[0029] These consensus sequences have been refined by comparison with the sequences present in certain regulatory genes from drosophila, xenopus, and human followed by point mutation, expression, and assay for activity. This approach has been successful in producing several active totally synthetic constructs not found in nature (as far as applicants are aware) which have true osteogenic activity.

[0030] These discoveries enable the construction of DNAs encoding totally novel, non-native protein constructs which individually and combined are capable of producing true endochondral bone. The DNAs may be expressed using well established recombinant DNA technologies in procaryotic or eucaryotic host cells, and the expressed proteins may be oxidized and refolded in vitro if necessary for biological activity.

[0031] The design and production of such biosynthetic proteins, the nature of the matrix, and other material aspects concerning the nature, utility, how to make, and how to use the subject matter claimed herein will be further understood from the following, which constitutes the best method currently known for practicing the various aspects of the invention.

CONSENSUS SEQUENCE DESIGN

[0032] A synthetic consensus gene shown in FIGURE 4 was designed to encode a consensus protein based on amino acid predictions from homology with the TGF-beta gene family. The designed concensus sequence was then constructed using known techniques involving assembly of oligonucleotides manufactured in a DNA synthesizer.

[0033] Tryptic peptides derived from Bovine Osteogenic Protein isolated by applicants and sequenced by Edman degradation provided amino acid sequences that showed strong homology with the Drosophila DPP protein sequence (as inferred from the gene), the Xenopus VG1 protein, and somewhat less homology to inhibin and TGF-beta, as demonstrated below in TABLE 1.

TABLE 1

protein	amino acid sequence	homology
(BOP)	SFDAYYCSGACQFPS	(9/15 matches)
	***** * * **	
(DPP)	GYDAYYCHGKCPFFL	
(BOP)	SFDAYYCSGACQFPS	(6/15 matches)
	* ** * * *	
(Vgl)	GYMANYCYGECPYPL	
(BOP)	SFDAYYCSGACQFPS	(5/15 matches)
	* ** * *	
(inhibin)	GYHANYCEGECPSHI	
(BOP)	SFDAYYCSGACQFPS	(4/15 matches)
	* * * *	
(TGF-beta)	GYHANFCLGPCPYIW	
(BOP)	K/RACCVPTELSAISMLYLDEN	(12/20 matches)
	***** * **** * *	
(Vgl)	LPCCVPTKMSPISMLFYDNN	
(BOP)	K/RACCVPTELSAISMLYLDEN	(12/20 matches)
	* ***** * **** *	
(inhibin)	KSCCVPTKLRPMSMLYYDDG	
(BOP)	K/RACCVPTELSAISMLYLDE	(6/19 matches)
	**** * *	
(TGF-beta)	APCCVPQALEPLPIVYYVG	
(BOP)	K/RACCVPTELSAISMLYLDEN	(12/20 matches)
	******* * ****	
(DPP)	KACCVPTQLDSVAMLYLNDQ	

(BOP)	LYVDF	
	*****	(5/5 matches)
(DPP)	LYVDF	

(BOP)	LYVDF	
	*** *	(4/5 matches)
(Vgl)	LYVEF	

(BOP)	LYVDF	
	** **	(4/5 matches)
(TGF-beta)	LYIDF	

(BOP)	LYVDF	
	* *	(2/5 matches)
(inhibin)	FFVSF	

*-match

[0034] In determining an appropriate amino acid sequence of an osteogenic protein (from which the nucleic acid sequence can be determined), the following points were considered: (1) the amino acid sequence determined by Edman degradation of natural source osteogenic protein tryptic fragments is ranked highest as long as it has a strong signal and shows homology or conservative changes when aligned with the other members of the gene family; (2) where the sequence matches for all four proteins, it is used in the synthetic gene sequence; (3) matching amino acids in DPP and Vgl are used; (4) If Vgl or DPP diverged but either one were matched by inhibin or by TGF-beta, this matched amino acid is chosen; (5) where all sequences diverged, the DPP sequence is initially chosen, with a later plan of creating the Vgl sequence by mutagenesis kept as a possibility. In addition, the consensus sequence is designed to preserve the disulfide crosslinking and the apparent structural homology among the related proteins.

RECOMBINANT OSTEOGENIC PROTEIN CONSTRUCTS

[0035] This approach resulted in the production of novel recombinant proteins capable of inducing formation of cartilage and endochondral bone comprising a protein structure analogous to or duplicative of the functional domain of the naturally sourced material. The amino acid sequences encoded by the consensus DNA sequences were derived from a family of natural proteins implicated in tissue development. These gene products/proteins are known to exist in active form as dimers and are, in general, processed from a precursor protein to produce an active C-terminal domain of the precursor.

[0036] The recombinant osteogenic/chondrogenic proteins are "novel" in the sense that, as far as applicants are aware, they do not exist in nature or, if they do exist, have never before been associated with bone or cartilage formation. The approach to design of these proteins is to employ amino acid sequences, found in the native OP isolates, in polypeptide structures are patterned after certain proteins reported in the literature, or the amino acid sequences inferred from DNAs reported in the literature. Thus, using the design criteria set forth above, and refining the amino acid sequence as more protein sequence information was learned, a series of synthetic proteins were designed with the hope and intent that they might have osteogenic or chondrogenic activity when tested in the bioassay system disclosed below.

[0037] It was noted, for example, that DPP from drosophila, VG1 from Xenopus, the TGF beta family of proteins, and to a lesser extent, alpha and beta inhibins, had significant homologies with certain of the sequences derived from

the naturally sourced OP product. (FIGURE 1.) Study of these proteins led to the realization that a portion of the sequence of each had a structural similarity observable by analysis of the positional relationship of cysteines and other amino acids which have an important influence on three dimensional protein conformation. It was noted that a region of these sequences had a series of seven cysteines, placed very nearly in the same relative positions, and certain other amino acids in sequence as set forth below:

```
         10        20        30        40        50
CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX
         60        70        80        90       100
QXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX
```

wherein each X independently represents an amino acid. Expression experiments of two of these constructs demonstrate activity. Expression experiments with constructs patterned after this template amino acid sequence with a shorter sequence having only six cysteines also show activity:

```
         10        20        30        40        50
    LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX
         60        70        80        90       100
QXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX
```

wherein each X independently represents an amino acid. Within these generic structures are a multiplicity of specific sequences which have osteogenic or chondrogenic activity. Preferred structures are those having the amino acid sequence:

```
         10        20        30        40        50
CKRHPLYVDFRDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNSTNHAIV
 RKRS K S S L  QE VIS E FD Y  E A AY MPESMKAS   VI
   KE F E K I  DN     L    N  S   Q  ITK F P    TL
    Q     A    S      K
         60        70        80        90       100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
 SI HAI SEQV EP  A EQMNSLAI FFNDQDK I RK EE T DA H H
    RF    T   S     K DPV V  Y N S     H RN   RS
     N    S                      K       P     E
```

wherein, in each positibn where more than one amino acid is shown, any one of the amino acids shown may be used. Novel active proteins also are defined by amino acid sequences comprising an active domain beginning at residue number 6 of this sequence, i.e, omitting the N terminal CXXXX, or omitting any of the preferred specific combinations such as CKRHP, CRRKQ, CKRHE, etc, resulting in a construct having only six cysteine residues. After this work, PCT 87/01537 was published, and it was observed that the proteins there identified as BMPII a and b and BMPIII each included a region embodying this generic structure. These proteins were not demonstrated to be osteogenic in the published application. However, applicants discovered that a subpart of the amino acid sequence of these proteins, properly folded, and implanted as set forth herein, is active. These are disclosed herein as CBMPIIa, CBMPIIb, and CBMPIII. Also, applicants retrieved a previously unreported gene by probing a human genomic DNA library with COPO. This protein was designated OP1. It comprises a region exhibiting the same generic structure.

[0038] Thus, the preferred osteogenic proteins are expressed from recombinant DNA and comprise amino acid sequences including any of the following sequences:

```
        1       10         20        30         40
Vgl     CKKRHLYVEFK-DVGWQNWVIAPQGYMANYCYGECPYPLTE
                   50         60         70
        ILNGSN--H-AILQTLVHSIEPED-IPLPCCVPTKMSP
           80         90        100
        ISMLFYDNNDNVVLRHYENMAVDECGCR

        1       10         20        30         40
DPP     CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD
                   50         60         70
        HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS
           80         90        100
        VAMLYLNDQSTVVLKNYQEMTVVGCGCR

        1       10         20        30         40
OP1          LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
                   50         60         70
        YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
           80         90        100
        ISVLYFDDSSNVILKKYRNMVVRACGCH

                                                -5
                                              HQRQA
        1       10         20        30         40
OP1     CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
                   50         60         70
        YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
           80         90        100
        ISVLYFDDSSNVILKKYRNMVVRACGCH

        1       10         20        30         40
CBMP-2a CKRHPLYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD
                   50         60         70
        HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTELSA
           80         90        100
        ISMLYLDENEKVVLKNYQDMVVEGCGCR
```

```
           1       10         20         30         40
CBMP-2b    CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPFPLAD
                      50         60         70
           HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA
              80         90       100
           ISMLYLDEYDKVVLKNYQEMVVEGCGCR

           1       10         20         30         40
CBMP-3     CARRYLKVDFA-DIGWSEWIISPKSFDAYYCSGACQFPMPK
                      50         60         70
           SLKPSN--H-ATIQSIVRAVGVVPGIPEPCCVPEKMSS
              80         90       100
           LSILFFDENKNVVLKVYPNMTVESCACR

           1       10         20         30         40
COP1            LYVDFQRDVGWDDWIIAPVDFDAYYCSGACQFPSAD
                      50         60         70
           HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
              80         90       100
           ISMLYLDENSTVVLKNYQEMTVVGCGCR

           1       10         20         30         40
COP3            LYVDFQRDVGWDDWIVAPPGYQAFYCSGACQFPSAD
                      50         60         70
           HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
              80         90       100
           ISMLYLDENEKVVLKNYQEMVVEGCGCR

           1       10         20         30         40
COP4            LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD
                      50         60         70
           HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
              80         90       100
           ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

```
                1         10          20          30          40
COP5                LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPFPLAD
                               50          60          70
                HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
                   80          90         100
                ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

```
                1         10          20          30          40
COP7                LYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD
                               50          60          70
                HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
                   80          90         100
                ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

```
                                                  10
                                              PKHHSQRARKKNKN
                1         10          20          30          40
COP16           CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPFPLAD
                               50          60          70
                HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
                   80          90         100
                ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

[0039] As shown in FIGURE 1, these sequences have considerable homology with the alpha and beta inhibins, three forms of TGF beta, and MIS.

Gene Preparation

[0040] The synthetic genes designed to express the proteins as described above preferably are produced by assembly of chemically synthesized oligonucleotides. 15-100mer oligonucleotides may be synthesized on a Biosearch DNA Model 8600 Synthesizer, and purified by polyacrylamide gel electrophoresis (PAGE) in Tris-Borate-EDTA buffer (TBE). The DNA is then electroeluted from the gel. Overlapping oligomers may be phosphorylated by T4 polynucleotide kinase and ligated into larger blocks which may also be purifed by PAGE. Natural gene sequences and cDNAs also may be used for expression.

Expression

[0041] The genes can be expressed in appropriate prokaryotic hosts such as various strains of E. coli and also in bacillus, yeasts, and various animal cells such as CHO, myeloma, etc. Generally, expression may be achieved using many cell types and expression systems well known to those skilled in the art. If the gene is to be expressed in E. coli, it must first be cloned into an expression vector. An expression vector (FIGURE 2A) based on pBR322 and containing a synthetic trp promoter operator and the modified trp LE leader can be opened at the EcoRI and PSTI restriction sites, and a FB-FB COP1, COP3, COPS, and COP7 gene fragments (FIGURE 2B) can be inserted between these sites, where FB is fragment B of Staphylococcal Protein A. The expressed fusion protein results from attachment of the COP gene to a fragment encoding FB. The COP protein is joined to the leader protein via a hinge region having the sequence Asp-Pro-Asn-Gly. This hinge permits chemical cleavage of the fusion protein with dilute acid at the asp-pro site or cleavage at Asn-Gly with hydroxylamine, resulting in release of the COP protein. For COP16 and OP1, the proteins are expressed as fusion products, using the modified trp-LE as a leader.

Production of Active Proteins

[0042] The following procedure was followed for production of active recombinant proteins. E. coli cells containing the fusion proteins were lysed. The fusion proteins were purified by differential solubilization. In the case of the COP1, 3, 4, 5, and 7 fusion proteins, cleavage was with dilute acid, and the resulting cleavage products were passed through a Sephacryl-200HR column. The Sephacryl column separated most of the uncleaved fusion products from the COP1, 3, 4, 5, and 7 analogs. In the case of the COP16 or OP1 fusion protein, cleavage was with a more concentrated acid, and an SP-Trisacryl column was used as an additional purification step. The COP or OP fractions were then subjected to HPLC on a semi-prep C-18 column.

[0043] Initial conditions for refolding of COP analogs or OP1 were at pH 8.0 using Tris, Gu-HCl, dithiothreitol. Final conditions for refolding of COP analogs were at pH 8.0 using Tris, oxidized glutathione, and lower amounts of Gu-HCl and dithiothreitol. Alternatively, the COP or OP1 proteins are suspended in 50 mM HCl, 6 M guanidine-HCl, pH 8.0, for 18 hours at 4°C. Refolding may not be required if the proteins are expressed in animal cells.

Production of Antisera

[0044] Antisera to COP7 and COPS were produced in New Zealand white rabbits. Western blots demonstrate that the antisera react with COP7 and COPS preparations. Antisera to COP7 has been tested for reactivity to naturally sourced bovine osteogenic protein samples. Western blots show a clear reaction with the 30 kD protein and, when reduced, with the 16 kD subunit. The immunoreactive species appears as a closely-spaced doublet in the 16 kD subunit region, similar to the 16 kD doublet seen in Con A blots.

MATRIX PREPARATION

General Consideration of Matrix Properties

[0045] The carrier described in the bioassay section, infra, may be replaced by either a biodegradable-synthetic or synthetic-inorganic matrix (e.g., HAP, collagen, tricalcium phosphate, or polylactic acid, polyglycolic acid and various copolymers thereof). Also xenogeneic bone may be used if pretreated as described below.

[0046] Studies have shown that surface charge, particle size, the presence of mineral, and the methodology for combining matrix and osteogenic protein all play a role in achieving successful bone induction. Perturbation of the charge by chemical modification abolishes the inductive response. Particle size influences the quantitative response of new bone; particles between 75 and 420 μm elicit the maximum response. Contamination of the matrix with bone mineral will inhibit bone formation. Most importantly, the procedures used to formulate osteogenic protein onto the matrix are extremely sensitive to the physical and chemical state of both the osteogenic protein and the matrix.

[0047] The sequential cellular reactions at the interface of the bone matrix/OP implants are complex. The multistep cascade includes: binding of fibrin and fibronectin to implanted matrix, chemotaxis of cells, proliferation of fibroblasts, differentiation into chondroblasts, cartilage formation, vascular invasion, bone formation, remodeling, and bone marrow differentiation.

[0048] A successful carrier for osteogenic protein must perform several important functions. It must bind osteogenic protein and act as a slow release delivery system, accommodate each step of the cellular response during bone development, and protect the osteogenic protein from nonspecific proteolysis. In addition, selected materials must be biocompatible in vivo and biodegradable; the carrier must act as a temporary scaffold until replaced completely by new bone. Biocompatibility requires that the matrix not induce significant inflammation when implanted and not be rejected by the host animal. Biodegradability requires that the matrix be slowly absorbed by the body of the host during development of new bone or cartilage. Polylactic acid (PLA), polyglycolic acid (PGA), and various combinations have different dissolution rates in vivo. In bones, the dissolution rates can vary according to whether the implant is placed in cortical or trabecular bone.

[0049] Matrix geometry, particle size, the presence of surface charge, and porosity or the presence of interstices among the particles of a size sufficient to permit cell infiltration, are all important to successful matrix performance. It is preferred to shape the matrix to the desired form of the new bone and to have dimensions which span non-union defects. Rat studies show that the new bone is formed essentially having the dimensions of the device implanted.

[0050] The matrix may comprise a shape-retaining solid made of loosely adhered particulate material, e.g., with collagen. It may also comprise a molded, porous solid, or simply an aggregation of close-packed particles held in place by surrounding tissue. Masticated muscle or other tissue may also be used. Large allogeneic bone implants can act as a carrier for the matrix if their marrow cavities are cleaned and packed with particles and the dispersed osteogenic protein.

Preparation of Biologically Active Allogenic Matrix

[0051] Demineralized bone matrix is prepared from the dehydrated diaphyseal shafts of rat femur and tibia as described herein to produce a bone particle size which pass through a 420 μm sieve. The bone particles are subjected to dissociative extraction with 4 M guanidine-HCl. Such treatment results in a complete loss of the inherent ability of the bone matrix to induce endochondral bone differentiation. The remaining insoluble material is used to fabricate the matrix. The material is mostly collagenous in nature, and upon implantation, does not induce cartilage and bone. All new preparations are tested for mineral content and false positives before use. The total loss of biological activity of bone matrix is restored when an active osteoinductive protein fraction or a pure protein is reconstituted with the biologically inactive insoluble collagenous matrix. The osteoinductive protein can be obtained from any vertebrate, e.g., bovine, porcine, monkey, or human, or produced using recombinant DNA techniques.

Preparation of Deglycosylated Bone Matrix for Use in Xenogenic Implant

[0052] When osteogenic protein is reconstituted with collagenous bone matrix from other species and implanted in rat, no bone is formed. This suggests that while the osteogenic protein is xenogenic (not species specific), while the matrix is species specific and cannot be implanted cross species perhaps due to intrinsic immunogenic or inhibitory components. Thus, heretofore, for bone-based matrices, in order for the osteogenic protein to exhibit its full bone inducing activity, a species specific collagenous bone matrix was required.

[0053] The major component of all bone matrices is Type I collagen. In addition to collagen, extracted bone includes non-collagenous proteins which may account for 5% of its mass. Many non-collagenous components of bone matrix are glycoproteins. Although the biological significance of the glycoproteins in bone formation is not known, they may present themselves as potent antigens by virtue of their carbohydrate content and may constitute immunogenic and/or inhibitory components that are present in xenogenic matrix.

[0054] It has now been discovered that a collagenous bone matrix may be used as a carrier to effect bone inducing activity in xenogenic implants, if one first removes the immunogenic and inhibitory components from the matrix. The matrix is deglycosglated chemically using, for example, hydrogen fluoride to achieve this purpose.

[0055] Bovine bone residue prepared as described above is sieved, and particles of the 74-420 μM are collected. The sample is dried in vacuo over P_2O_5, transferred to the reaction vessel and anhydrous hydrogen fluoride (HF) (10-20 ml/g of matrix) is then distilled onto the sample at -70°C. The vessel is allowed to warm to 0°C. and the reaction mixture is stirred at this temperature for 120 min. After evaporation of the HF in vacuo, the residue is dried thoroughly in vacuo over KOH pellets to remove any remaining traces of acid.

[0056] Extent of deglycosylation can be determined from carbohydrate analysis of matrix samples taken before and after treatment with HF, after washing the samples appropriately to remove non-covalently bound carbohydrates.

[0057] The deglycosylated bone matrix is next treated as set forth below:

1) suspend in TBS (Tris-buffered Saline) lg/200 ml and stir at 4°C for 2 hrs; or in 6 M urea, 50 mM Tris-HCl, 500 mM NaCl, pH 7.0 (UTBS) and stir at RT for 30 min;

2) centrifuge and wash with TBS or UTBS as in step 1; and

3) centrifuge; discard supernatant; water wash residue; and then lyophilize.

FABRICATION OF DEVICE

[0058] Fabrication of osteogenic devices using any of the matrices set forth above with any of the osteogenic proteins described above may be performed as follows.

A. Ethanol precipitation

[0059] In this procedure, matrix is added to osteogenic protein in guanidine-HCl. Samples are vortexed and incubated at a low temperature. Samples are then further vortexed. Cold absolute ethanol is added to the mixture which is then stirred and incubated. After centrifugation (microfuge high speed) the supernatant is discarded. The reconstituted matrix is washed with cold concentrated ethanol in water and then lyophilized.

B. Acetonitrile Trifluoroacetic Acid Lyophilization

[0060] In this procedure, osteogenic protein in an acetonitrile trifluroacetic acid (ACN/TFA) solution is added to the

carrier. Samples are vigorously vortexed many times and then lyophilized.

C. Urea Lyophilization

[0061] For those proteins that are prepared in urea buffer, the protein is mixed with the matrix, vortexed many times, and then lyophilized. The lyophilized material may be used "as is" for implants.

IN VIVO RAT BIOASSAY

[0062] Several of the synthetic proteins have been incorporated in matrices to produce osteogenic devices, and assayed in rat for endochondral bone. Studies in rats show the osteogenic effect to be dependent on the dose of osteogenic protein dispersed in the osteogenic device. No activity is observed if the matrix is implanted alone. The following sets forth guidelines for how the osteogenic devices disclosed herein can be assayed for evaluating protein constructs and matrices for biological activity.

A. Subcutaneous Implantation

[0063] The bioassay for bone induction as described by Sampath and Reddi (Proc. Natl. Acad. Sci. USA (1983) 80: 6591-6595), herein incorporated by reference, is used to assess endochondral bone differentiation activity. This assay consists of implanting the test samples in subcutaneous sites in allogenic recipient rats under ether anesthesia. Male Long-Evans rats, aged 28-32 days, were used. A vertical incision (1 cm) is made under sterile conditions in the skin over the thoracic region, and a pocket is prepared by blunt dissection. Approximately 25 mg of the test sample is implanted deep into the pocket and the incision is closed with a metallic skin clip. The day of implantation is designated as day one of the experiment. Implants were removed on day 12. The heterotropic site allows for the study of bone induction without the possible ambiguities resulting from the use of orthotopic sites.

B. Cellular Events

[0064] The implant model in rats exhibits a controlled progression through the stages of matrix induced endochondral bone development including: (1) transient infiltration by polymorphonuclear leukocytes on day one; (2) mesenchymal cell migration and proliferation on days two and three; (3) chondrocyte appearance on days five and six; (4) cartilage matrix formation on day seven; (5) cartiliage calcification on day eight; (6) vascular invasion, appearance of osteoblasts, and formation of new bone on days nine and ten; (7) appearance of osteoblastic and bone remodeling and dissolution of the implanted matrix on days twelve to eighteen; and (8) hematopoietic bone marrow differentiation in the ossicle on day twenty-one. The results show that the shape of the new bone conforms to the shape of the implanted matrix.

C. Histological Evaluation

[0065] Histological sectioning and staining is preferred to determine the extent of osteogenesis in the implants. Implants are fixed in Bouins Solution, embedded in parafilm, cut into 6-8 mm sections. Staining with toluidine blue or hemotoxylin/eosin demonstrates clearly the ultimate development of endochondrial bone. Twelve day implants are usually sufficient to determine whether the implants show bone inducing activity.

D. Biological Markers

[0066] Alkaline phosphatase activity may be used as a marker for osteogenesis. The enzyme activity may be determined Spectrophotometrically after homogenization of the implant. The activity peaks at 9-10 days in vivo and thereafter slowly declines. Implants showing no bone development by histology should have little or no alkaline phosphatase activity under these assay conditions. The assay is useful for quantitation and obtaining an estimate of bone formation very quickly after the implants are removed from the rat. Alternatively the amount of bone formation can be determined by measuring the calcium content of the implant.

[0067] The osteogenic activity due to osteogenic protein is represented by "bone forming units". One bone forming unit represents the amount of protein that is needed for half maximal bone forming activity as compared to rat demineralized bone matrix as control and determined by calcium content of the implant on day 12.

[0068] Devices that contained only rat carrier show complete absence of new bone formation. The implant consists of carrier rat matrix and surrounding mesenchymal cells. Again, the devices that contained rat carrier and not correctly folded (or biologically inactive) recombinant protein also showed complete absence of bone formation. These implants are scored as cartilage formation (-) and bone formation (-). The endochondral bone formation activity is scored as

zero percent (0%) (FIGURE 3A).

[0069] Implants included biologically active recombinant protein, however, showed evidence of endochondral bone formation. Histologically they showed new cartilage and bone formation.

[0070] The cartilage formation is scored as (+) by the presence of metachromatically stained chondrocytes in the center of the implant, as (++) by the presence of numerous chondrocytes in many areas of the implant and as (+++) by the presence of abundant chondrocytes forming cartilage matrix and the appearance of hypertrophied chondrocytes accompanying cartilage calcification (FIGURE 3B).

[0071] The bone formation is scored as (+) by the presence of osteoblast surrounding vascular endothelium forming new matrix, as (++) by the formation of bone due to osteoblasts (as indicated by arrows) and further bone remodeling by the appearance of osteoclasts in opposition to the newly formed bone matrix. Vascular invasion is evident in these implants (FIGURE 3B). Formation is scored as (+++) by the presence of extensive remodeled bone which results in the formation of ossicles.

[0072] The overall bone inducing activity due to recombinant protein is represented as percent response of endochondral bone formation (see TABLE 2 below).

TABLE 2

HISTOLOGICAL EVALUATION OF RECOMBINANT BONE INDUCTIVE PROTEINS			
Sample No.	Implanted Protein	Cartilage Formation	Bone Formation
260-54	COP-5	+++	++
279-5	COP-5	++	+
285-13	COP-5	+++	++
277-7	COP-7	+++	++
277-8	COP-7	+++	++
277-9	COP-7	++	+
285-14	COP-7	+++	++
285-24	COP-7	++	+
285-25	COP-7	++	++
314-6	COP-16	+++	+++
314-15	COP-16	++	+
314-16	COP-16	++	+
314-12	OP-1	++	+

[0073] The invention relates to an osteogenic device for implantation in a mammal, said device comprising a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains, each of which comprises an amino acid sequence sufficiently duplicative of the sequence of COP5, COP7, COP16, or OP1 such that said protein is capable of inducing endochondral bone formation in association with said matrix when implanted in a mammal.

[0074] Also covered by the invention is a device for implantation in a mammal, said device comprising a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains, each of which has less than about 200 amino acids, and each of which comprises a sequence sufficiently duplicative of the sequence of COP5, COP7, COP16, or OP1 such that said protein is capable of inducing cartilage formation in association with said matrix when implanted in a mammal.

[0075] The sequence may comprise

```
          10         20         30         40         50
CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX
          60         70         80         90        100
QXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX
```

wherein each X independently represents an amino acid; or

```
        10        20        30        40        50
     LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX
        60        70        80        90       100
QXXVXXXNXXXXPXXCCXPXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX
```

wherein each X independently represents an amino acid; or

```
          10        20        30        40        50
 CKRHPLYVDFRDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNSTNHAIV
  RKRS K S S L  QE VIS E FD Y  E A AY MPESMKAS   VI
    KE F E K I  DN     L   N  S   Q  ITK F P    TL
     Q    A   S      K
          60        70        80        90       100
 QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
  SI HAI SEQV EP  A EQMNSLAI FFNDQDK I RK EE T DA H H
     RF    T   S    K DPV V  Y N S    H RN   RS
      N    S                  K       P    E
```

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position; or

```
        10        20        30        40        50
     LYVDFRDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNSTNHAIV
      K S S L  QE VIS E FD Y  E A AY MPESMKAS   VI
      F E K I  DN     L   N  S   Q  ITK F P    TL
          A   S      K
        60        70        80        90       100
 QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
  SI HAI SEQV EP  A EQMNSLAI FFNDQDK I RK EE T DA H H
     RF    T   S    K DPV V  Y N S    H RN   RS
      N    S                  K       P    E
```

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position; or

```
               1        10        20        30        40
Vgl            CKKRHLYVEFK-DVGWQNWVIAPQGYMANYCYGECPYPLTE
                         50        60        70
               ILNGSN--H-AILQTLVHSIEPED-IPLPCCVPTKMSP
                   80        90       100
               ISMLFYDNNDNVVLRHYENMAVDECGCR
```

or

```
              1         10         20         30         40
DPP           CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD
                                  50         60         70
              HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS
                 80         90         100
              VAMLYLNDQSTVVLKNYQEMTVVGCGCR
```

or

```
              1         10          20         30         40
OP1                LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
                                  50         60         70
              YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
                 80         90         100
              ISVLYFDDSSNVILKKYRNMVVRACGCH
```

or

```
                                                    -5
                                                 HQRQA
              1         10          20         30         40
OP1           CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
                                  50         60         70
              YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
                 80         90         100
              ISVLYFDDSSNVILKKYRNMVVRACGCH
```

or

```
              1         10         20         30         40
CBMP-2a       CKRHPLYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD
                                  50         60         70
              HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTELSA
                 80         90         100
              ISMLYLDENEKVVLKNYQDMVVEGCGCR
```

or

```
              1         10         20         30         40
CBMP-2b       CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPFPLAD
                                  50         60         70
              HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA
                 80         90         100
              ISMLYLDEYDKVVLKNYQEMVVEGCGCR
```

or

```
        1         10         20          30          40
CBMP-3  CARRYLKVDFA-DIGWSEWIISPKSFDAYYCSGACQFPMPK
                  50          60          70
        SLKPSN--H-ATIQSIVRAVGVVPGIPEPCCVPEKMSS
             80          90         100
        LSILFFDENKNVVLKVYPNMTVESCACR
```

or

```
        1          10          20          30          40
COP1          LYVDFQRDVGWDDWIIAPVDFDAYYCSGACQFPSAD
                   50          60          70
        HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
             80          90          100
        ISMLYLDENSTVVLKNYQEMTVVGCGCR
```

or

```
        1          10          20          30          40
COP3          LYVDFQRDVGWDDWIVAPPGYQAFYCSGACQFPSAD
                   50          60          70
        HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
             80          90          100
        ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

or

```
        1          10          20          30          40
COP4          LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD
                   50          60          70
        HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
             80          90          100
        ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

or

```
        1          10          20          30          40
COP5          LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPFPLAD
                   50          60          70
        HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
             80          90          100
        ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

or

```
              1          10         20         30        40
COP7                LYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD
                              50         60         70
              HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
                  80        90         100
              ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

or

```
                                                   10
                                                PKHHSQRARKKNKN
              1         10         20          30        40
COP16         CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPFPLAD
                              50         60          70
              HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
                  80        90         100
              ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

[0076] The device of the invention may comprise an osteogenic protein comprising a pair of separate polypeptide chains.

[0077] The invention also relates to an osteogenic protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains each of which comprises a sequence sufficiently duplicative of the sequence of COP5, COP7, COP16, or OP1 such that said protein is capable of inducing endochondral bone formation in association with a matrix when implanted in a mammal.

[0078] In another embodiment, the invention relates to a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains less than about 200 amino acids long and comprising a sequence sufficiently duplicative of the sequence of COP5, COP7, COP16, or OP1 such that said protein is capable of inducing cartilage formation in association with a matrix when implanted in a mammal.

[0079] The protein may be unglycosylated, and may comprise the amino acid sequences:

```
          10         20         30         40         50
CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX
          60         70         80         90        100
QXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX
```

or

```
     10         20         30         40         50
LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX
     60         70         80         90        100
QXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX
```

wherein each X independently represents an amino acid.

[0080] The protein of the invention may comprise the amino acid sequences:

```
           10          20          30          40          50
CKRHPLYVDFRDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNSTNHAIV
 RKRS K S S L  QE VIS E FD Y  E A AY MPESMKAS   VI
   KE F E K I  DN      L    N  S   Q  ITK F P    TL
    Q    A    S      K
           60          70          80          90         100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
 SI HAI SEQV EP  A EQMNSLAI FFNDQDK I RK EE T DA H H
    RF    T   S     K DPV V  Y N S     H RN   RS
     N    S                     K        P     E
```

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

[0081] The protein of the invention may comprise the amino acid sequences:

```
           10          20          30          40          50
      LYVDFRDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNSTNHAIV
       K S S L  QE VIS E FD Y  E A AY MPESMKAS   VI
       F E K I  DN      L    N  S   Q  ITK F P    TL
          A    S      K
           60          70          80          90         100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
 SI HAI SEQV EP  A EQMNSLAI FFNDQDK I RK EE T DA H H
    RF    T   S     K DPV V  Y N S     H RN   RS
     N    S                     K        P     E
```

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

[0082] The protein of the invention may comprise the amino acid sequences:

```
           1        10         20         30         40
Vgl        CKKRHLYVEFK-DVGWQNWVIAPQGYMANYCYGECPYPLTE
                       50         60         70
           ILNGSN--H-AILQTLVHSIEPED-IPLPCCVPTKMSP
              80         90         100
           ISMLFYDNNDNVVLRHYENMAVDECGCR
```

or

```
           1        10         20         30         40
DPP        CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD
                       50         60         70
           HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS
              80         90         100
           VAMLYLNDQSTVVLKNYQEMTVVGCGCR
```

or

```
                1           10           20           30           40
OP1                   LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
                              50           60           70
          YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
             80           90           100
          ISVLYFDDSSNVILKKYRNMVVRACGCH
```

or

```
                                                          -5
                                                        HQRQA
          1           10           20           30           40
OP1       CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
                        50           60           70
          YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
             80           90           100
          ISVLYFDDSSNVILKKYRNMVVRACGCH
```

or

```
          1           10           20           30           40
CBMP-2a   CKRHPLYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD
                        50           60           70
          HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTELSA
             80           90           100
          ISMLYLDENEKVVLKNYQDMVVEGCGCR
```

or

```
          1           10           20           30           40
CBMP-2b   CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPFPLAD
                        50           60           70
          HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA
             80           90           100
          ISMLYLDEYDKVVLKNYQEMVVEGCGCR
```

or

```
           1         10          20          30          40
CBMP-3     CARRYLKVDFA-DIGWSEWIISPKSFDAYYCSGACQFPMPK
                          50          60          70
           SLKPSN--H-ATIQSIVRAVGVVPGIPEPCCVPEKMSS
               80          90          100
           LSILFFDENKNVVLKVYPNMTVESCACR
```

or

```
           1         10          20          30          40
COP1           LYVDFQRDVGWDDWIIAPVDFDAYYCSGACQFPSAD
                          50          60          70
           HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
               80          90          100
           ISMLYLDENSTVVLKNYQEMTVVGCGCR
```

or

```
           1         10          20          30          40
COP3           LYVDFQRDVGWDDWIVAPPGYQAFYCSGACQFPSAD
                          50          60          70
           HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
               80          90          100
           ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

or

```
           1         10          20          30          40
COP4           LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD
                          50          60          70
           HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
               80          90          100
           ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

or

```
                1          10          20          30          40
COP5                 LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPFPLAD
                             50          60          70
                HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
                    80          90          100
                ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

or

```
                1          10          20          30          40
COP7                 LYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD
                             50          60          70
                HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
                    80          90          100
                ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

or

```
                                                   -10
                                              PKHHSSRARKKNKN
                1          10          20          30          40
COP16           CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPFPLAD
                             50          60          70
                HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
                    80          90          100
                ISMLYLDENEKVVLKNYQEMVVEGCGCR
```

[0083] The protein of the invention may comprise the product of expression of a DNA in a procaryotic cell.

[0084] Also contemplated by the invcention is a cell line engineered to express the protein of the invention.

[0085] Preferably, the protein has a half maximum bone forming activity of about 20-25 ng per 25 mg of implant.

[0086] The invention also contemplates antibodies reactive with an epitope of the protein of the invention.

[0087] The device of the invention preferably includes a matrix comprising demineralized, deglycosylated, protein extracted, particulate, xenogenic bone, or demineralized, protein extracted, particulate, xenogenic bone treated with HF.

Claims

1. Osteogenic protein comprising a pair of polypeptide chains bonded in the unreduced state to form a homo- or heterodimeric species having a conformation such that the pair of polypeptide chains is capable of inducing endochondral bone formation when disposed within a matrix and implanted in a mammal, wherein the protein comprises a region containing six cysteine residues positioned in the relative positions shown in the sequence:

   LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXXQXXVX
   XXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX,

   wherein the letters indicate the amino acid residues of standard single letter code and each X represents any amino acid, with the proviso that dpp is excluded.

2. The protein of claim 1 wherein the protein comprises a region containing seven cysteine residues positioned in the relative positions shown in the sequence:

CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX
QXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX.

3. The protein of claim 1 or claim 2 which is a recombinant protein produced by expression in a host cell.

4. A process for producing active synthetic osteogenic protein as defined in claim 1, said protein comprising a pair of polypeptide chains disulphide bonded to produce a dimeric species, the process comprising the steps of:

(a) producing a consensus amino acid sequence by comparing amino acid sequences of proteins of the TGF-beta gene family, which consensus sequence preserves the disulphide crosslinking pattern present in the sequence:

(i) LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXN
HAXXQXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXL
XXYXXMXVXXCXCX;

or

(ii) CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXX
XXXXNHAXXQXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXX
XXVXLXXYXXMXVXXCXCX;

wherein the letters indicate the amino acid residues of standard single letter code and each X represents any amino acid;

(b) constructing a synthetic gene encoding the consensus amino acid sequence of step (a), for example by assembling chemically synthesised oligonucleotides;

(c) introducing the synthetic gene of step (b) into a prokaryotic or eukaryotic host cell;

(d) expressing the synthetic gene to produce a putative synthetic osteogenic protein;

(e) assaying the putative synthetic osteogenic protein to determine whether it is active in inducing endochondral bone formation when implanted in a mammal, and optionally

(f) repeating steps (a) to (e) (or mutagenizing the synthetic gene and repeating steps (c) to (e) if the putative synthetic osteogenic protein is inactive when so assayed.

5. The process of claim 4 wherein the consensus nucleic acid sequence comprises the sequence:

(a) LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXN
HAXXQXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXL
XXYXXMXVXXCXCX;

or

(b) CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXXQXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX;

wherein the letters indicated the amino acid residues of standard single letter code and each X represents any amino acid.

6. The process of any one of claims 4-5 wherein the synthetic gene of step (b) comprises a nucleotide sequence which encodes a synthetic polypeptide chain having the same number of cysteine residues in the same relative positions as COPS, COP7, COP16 or OP1.

7. The process of any one of claims 4-6 wherein the synthetic gene of step (b) comprises a nucleotide sequence which encodes a synthetic polypeptide chain sharing one or more amino acids at corresponding positions in any of the sequences: Vg1, DPP, OP1, CBMP-2a, CBMP-2b, CBMP-3, COP1, COP3, COP4, COPS, COP7 or COP16.

8. The process of any one of claims 4-7 wherein the synthetic gene of step (b) comprises a nucleotide sequence which encodes a synthetic polypeptide chain comprising the amino acid sequence:

(a)

```
       10        20       30        40         50
CKRHPLYVDFRDVGWNDWIVPPGYHAFYCHGECPFPLADHLNSTNHAIV
 RKRS K S S L QE VIS E FD Y  E A AY MPESMKAS   VI
   KE F E K I DN     L    N  S   Q  ITK F P    TL
    Q     A   S      K
        60        70        80        90       100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
 SI HAI SEQV EP  A EQMNSLAI FFNDQDK I RK EE T DA H H
    RF    T   S     K DPV V  Y N S     H RN   RS
     N    S                      K       P     E
```

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position; or

(b)

```
       10        20       30        40         50
    LYVDFRDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNSTNHAIV
      K S S L QE VIS E FD Y  E A AY MPESMKAS   VI
      F E K I DN     L    N  S   Q  ITK F P    TL
          A   S      K
        60        70        80        90         100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKKYQDMVVEGCGCR
 SI HAI SEQV EP  A EQMNSLAI FFNDQDK I RK EE T DA H H
    RF    T   S     X DPV V  Y N S     H RN   RS
     N    S                      K       P     E
```

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position; or

(c)

```
1      10         20        30        40
CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD
            50        60         70
 HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS
     80        90       100
 VAMLYLNDQSTVVLKNYQEMTVVGCGCR; or
```

(d)

```
1      10         20        30        40
    LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
            50        60         70
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
     80        90       100
ISVLYFDDSSNVILKKYRNMVVRACGCH; or
```

(e)

```
                                     -5
                                     HQRQA
1      10         20        30        40
CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
            50        60         70
 YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
     80        90       100
 ISVLYFDDSSNVILKKYRNMVVRACGCH; or
```

(f)

```
1         10              20             30             40
      LYVDFQRDVGWDDWIIAPVDFDAYYCSGACQFPSAD
                 50             60              70
HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
        80            90           100
ISMLYLDENSTVVLKNYQEMTVVGCGCR;   or
```

(g)

```
1         10              20             30             40
      LYVDFQRDVGWDDWIVAPPGYQAFYCSGACQFPSAD
                 50             60              70
HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
  80            90           100
ISMLYLDENEKVVLKNYQEMVVEGCGCR; or
```

(h)

```
1         10              20             30             40
      LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD
                 50             60              70
HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
      80            90           100
ISMLYLDENEKVVLKKYQEMVVEGCGCR.
```

9. The protein of any one of claims 1-3 wherein the protein comprises a synthetic polypeptide chain of less than 200 amino acids.

10. The process of any one of claims 4-8 wherein the protein comprises a synthetic polypeptide chain of less than 200 amino acids.

11. The protein of claim 3 wherein the host cell is a prokaryotic or eukaryotic host cell, for example E. coli, bacillus, yeasts or animal cells (e.g. CHO or myeloma).

12. The process of any one of claims 4-8 or 10 wherein the host cell is a prokaryotic or enkaryotic host cell, for example

E. coli, bacillus, yeasts or animal cells (e.g. CHO or myeloma).

13. The protein of any one of claims 1-3 wherein the protein is unglycosylated.

14. The process of any one of claims 4-8 or 10 wherein the protein is unglycosylated.

15. A composition comprising protein comprising a pair of polypeptide chains disulphide bonded to produce a dimeric species, the protein being as defined in claim 1, 2 or 3.

16. An osteogenic device comprising a matrix in which is disposed the protein of claim 1, 2 or 3.

17. An osteogenic device for implantation in a mammal, the device comprising: (a) a biocompatible, in vivo biodegradable matrix defining a scaffold of dimensions sufficient to permit the attachment, proliferation and differentiation of migratory progenitor cells from the body of said mammal, and (b) the protein of claim 1, 2 or 3 disposed in said matrix and accessible to said cells.

18. The device of claim 16 or claim 17 wherein said matrix comprises close-packed particulate matter having a particle size within the range of 70 to 850 µm (e.g. 70 to 420 µm).

19. The device of any one of claims 16-18 wherein said matrix comprises: (a) allogenic bone, e.g. demineralized, protein extracted, particulate, allogenic bone, (b) demineralized, protein extracted, particulate, deglycosylated xenogenic bone, (c) demineralized, protein extracted, particulate xenogenic bone treated with HF or a protease, (d) materials selected from collagen, hydroxyapatite, calcium phosphates (e.g. tricalcium phosphate) and polymers comprising glycolic acid and/or lactic acid monomers, (e) a shape-retaining solid of loosely adhered particulate material e.g. collagen, (f) a porous solid or (g) masticated tissue, e.g. muscle.

20. The device of any one of claims 16 to 19 disposed within the marrow cavity of allogenic bone.

21. The device of any of claims 16 to 20 for use in therapy, e.g. for inducing local cartilage and/or endochondral or heterotopic bone formation in a mammal by implanting the device in a mammal at a locus accessible to migratory progenitor cells.

22. The device of claim 21 for the formation of shaped heterotopic bone, wherein the shape of the heterotopic bone formed conforms to that of the implanted device.

23. Use of the protein of claim 1, 2 or 3 for the manufacture of: (a) a device according to any of claims 16 to 21, or (b) a medicament, the medicament or device being for inducing local cartilage and/or endochondral or heterotopic bone formation in a mammal by introducing the medicament or device in a mammal at a locus accessible to migratory progenitor cells for periodontal treatment, cartilage repair and for the treatment of osteoarthritis or to correct non-union fractures, acquired or congenital craniofacial and other skeletal or dental anomalies.

24. The use of claim 23 for the formation of shaped heterotopic bone, wherein the shape of the heterotopic bone formed conforms to that of the implanted device.

25. A synthetic nucleic acid molecule for use in the process of any one of claims 4 to 8.

26. A host cell for use in the process of any one of claims 4 to 8 which contains the synthetic nucleic acid molecule of claim 25.

27. The host cell of claim 26 which is a prokaryotic or eukaryotic host cell, for example E. coli, bacillus, yeasts or animal cells (e.g. CHO or myeloma).

28. An isolated DNA sequence which encodes the amino acid sequence of the protein as defined in any one of claims 1-3 or 9.

Patentansprüche

1. Osteogenes Protein, das zwei Polypeptidketten umfasst, die im nicht-reduzierten Zustand gebunden sind, so dass sie eine homo- oder heterodimere Spezies mit einer solchen Konformation bilden, dass die zwei Polypeptidketten dazu in der Lage sind, eine Ersatzknochenbildung zu induzieren, wenn sie in einer Matrix angeordnet und einem Säugetier implantiert werden, wobei das Protein einen Bereich umfasst, der sechs Cystein-Reste enthält, die in den in der Sequenz dargestellten relativen Positionen angeordnet sind:

   LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXXQXXVX
   XXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX,

   wobei die Buchstaben die Aminosäurereste im Standard Ein-Buchstaben-Code angeben und jedes X irgendeine Aminosäure repräsentiert, unter dem Vorbehalt, dass dpp ausgenommen ist.

2. Protein nach Anspruch 1,
   wobei das Protein einen Bereich umfasst, der sieben Cystein-Reste enthält, die in den in der Sequenz dargestellten relativen Positionen angeordnet sind:

   CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX
   QXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX.

3. Protein nach Anspruch 1 oder Anspruch 2,
   das ein rekombinantes Protein ist, das durch Expression in einer Wirtszelle hergestellt wird.

4. Verfahren zur Herstellung eines aktiven synthetischen osteogenen Proteins, wie in Anspruch 1 definiert, wobei das Protein zwei Polypeptidketten umfasst, die durch DisulfidBrücken verbunden sind, so dass eine dimere Spezies erzeugt wird, wobei das Verfahren die folgenden Schritte umfasst:

   (a) Erzeugen einer Aminosäure-Consensussequenz durch Vergleich von Aminosäuresequenzen von Proteinen der TGF-beta-Genfamilie, wobei die Consensussequenz das Disulfidvernetzungsmuster erhält, das in der folgenden Sequenz vorliegt:

   (i) LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXN
   HAXXQXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXL
   XXYXXMXVXXCXCX;

   oder

   (ii) CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXX
   XXXXNHAXXQXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXX
   XXVXLXXYXXMXVXXCXCX;

   wobei die Buchstaben die Aminosäurereste im Standard Ein-Buchstaben-Code anzeigen und jedes X irgendeine Aminosäure repräsentiert;

   (b) Konstruieren eines synthetischen Gens, das die Aminosäure-Consensussequenz von Schritt (a) kodiert,

beispielsweise durch Zusammenfügen von chemisch synthetisierten Oligonukleotiden;

(c) Einbringen des synthetischen Gens aus Schritt (b) in eine prokaryontische oder eukaryontische Wirtszelle;

(d) Exprimieren des synthetischen Gens zur Erzeugung eines putativen synthetischen osteogenen Proteins;

(e) Untersuchen des putativen synthetischen osteogenen Proteins, um zu bestimmen, ob es zur Induktion einer Ersatzknochenbildung wirksam ist, wenn es einem Säugetier implantiert wird, und wahlweise

(f) Wiederholen der Schritte (a) bis (e) (oder Mutagenisieren des synthetischen Gens und Wiederholen der Schritte (c) bis (e), wenn das putative synthetische osteogene Protein inaktiv ist, wenn es so geprüft wird).

5. Verfahren nach Anspruch 4,
wobei die Nukleinsäure-Consensussequenz die folgende Sequenz umfasst:

(a) LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXN
HAXXQXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXL
XXYXXMXVXXCXCX;

oder

(b) CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXX
XXXXNHAXXQXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXX
XXVXLXXYXXMXVXXCXCX;

wobei die Buchstaben die Aminosäurereste im Standard Ein-Buchstaben-Code angeben und jedes X irgendeine Aminosäure repräsentiert.

6. Verfahren nach einem oder mehreren der Ansprüche 4 bis 5,
wobei das synthetische Gen aus Schritt (b) eine Nukleotidsequenz umfasst, die eine synthetische Polypeptidkette mit derselben Anzahl an Cystein-Resten in denselben relativen Positionen kodiert, wie COP5, COP7, COP16 oder OP1.

7. Verfahren nach irgendeinem der Ansprüche 4 bis 6,
wobei das synthetische Gen aus Schritt (b) eine Nukleotidsequenz umfasst, die eine synthetische Polypeptidkette kodiert, die eine oder mehrere Aminosäuren an den entsprechenden Positionen in irgendeiner der Sequenzen: Vg1, DPP, OP1, CBMP-2a, CMBP-2b, CBMP-3, COP1, COP3, COP4, COP5, COP7 oder COP16, umfasst.

8. Verfahren nach einem der Ansprüche 4 bis 7,
wobei das synthetische Gen aus Schritt (b) eine Nukleotidsequenz umfasst, die eine synthetische Polypeptidkette kodiert, die die folgende Aminosäure umfasst:

(a)

```
          10        20        30        40        50
CKRHPLYVDFRDVGWNDWIVPPGYHAFYCHGECPFPLADHLNSTNHAIV
 RKRS K S S L QE VIS E FD Y  E A AY MPESMKAS   VI
   KE F E K I DN     L   N  S   Q  ITK F P    TL
     Q   A  S        K
          60        70        80        90        100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
 SI HAI SEQV EP  A EQMNSLAI FFNDQDK I RK EE T DA H H
    RF    T   S    K DPV V  Y N S      H RN   RS
    N     S                   K         P     E
```

wobei in jeder Position, in der mehr als eine Aminosäure dargestellt ist, irgendeine der dargestellten Aminosäuren in dieser Position vorliegen kann; oder

(b)

```
     10        20        30        40        50
LYVDFRDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNSTNHAIV
 K S S L  QE VIS E FD Y  E A AY MPESMKAS   VI
 F E K I  DN     L   N  S   Q  ITK F P    TL
     A    S      K
          60        70        80        90        100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKKYQDMVVEGCGCR
 SI HAI SEQV EP  A EQMNSLAI FFNDQDK I RK EE T DA H H
    RF    T   S    X DPV V  Y N S      H RN   RS
    N     S                   K         P     E
```

wobei in jeder Position, in der mehr als eine Aminosäure dargestellt ist, irgendeine der dargestellen Aminosäuren in dieser Position vorliegen kann; oder

(c)

```
1        10         20         30         40
CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD
              50        60          70
HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS
       80         90        100
VAMLYLNDQSTVVLKNYQEMTVVGCGCR;
```

oder

(d)

```
1         10             20            30            40
    LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
               50            60            70
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
     80           90           100
ISVLYFDDSSNVILKKYRNMVVRACGCH;
```

oder

(e)

```
                                                -5
                                              HQRQA
1         10             20            30            40
CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
               50            60            70
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
     80           90           100
ISVLYFDDSSNVILKKYRNMVVRACGCH;
```

oder

(f)

```
1         10             20            30            40
    LYVDFQRDVGWDDWIIAPVDFDAYYCSGACQFPSAD
               50            60            70
HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
     80           90           100
ISMLYLDENSTVVLKNYQEMTVVGCGCR;
```

oder

(g)

```
1     10        20        30        40
    LYVDFQRDVGWDDWIVAPPGYQAFYCSGACQFPSAD
           50        60         70
HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
 80        90        100
ISMLYLDENEKVVLKNYQEMVVEGCGCR;
```

oder

(h)

```
1     10        20        30        40
    LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD
           50        60         70
HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
     80        90        100
ISMLYLDENEKVVLKKYQEMVVEGCGCR.
```

9. Protein nach einem der Ansprüche 1 bis 3, wobei das Protein eine synthetische Polypeptidkette von weniger als 200 Aminosäuren umfasst.

10. Verfahren nach einem der Ansprüche 4 bis 8, wobei das Protein eine synthetische Polypeptidkette von weniger als 200 Aminosäuren umfasst.

11. Protein nach Anspruch 3, wobei die Wirtszelle eine prokaryontische oder eukaryontische Wirtszelle ist, beispielsweise E. coli, Bacillus, Hefen oder Tierzellen (beispielsweise CHO oder Myeloma).

12. Verfahren nach einem der Ansprüche 4 bis 8 oder 10, wobei die Wirtszelle eine prokaryontische oder eukaryontische Wirtszelle ist, beispielsweise E. coli, Bacillus, Hefen oder Tierzellen (beispielsweise CHO oder Myeloma).

13. Protein nach einem der Ansprüche 1 bis 3, wobei das Protein unglykosyliert ist.

14. Verfahren nach einem der Ansprüche 4 bis 8 oder 10, wobei das Protein unglykosyliert ist.

15. Zusammensetzung, die ein Protein umfasst, dass zwei Polypeptidketten, die durch Disulfidbrücken verbunden sind, umfasst, um eine dimere Spezies zu erzeugen, wobei das Protein wie in Anspruch 1, 2 oder 3 definiert ist.

16. Osteogene Vorrichtung, die eine Matrix umfasst, in der das Protein nach Anspruch 1, 2 oder 3 angeordnet ist.

17. Osteogene Vorrichtung zur Implantation in ein Säugetier, wobei die Vorrichtung Folgendes umfasst: (a) eine biokompatible, in vivo biologisch abbaubare Matrix, die ein Gerüst mit Abmessungen definiert, die ausreichend sind, um die Anlagerung, Proliferation und Differentiation von migratorischen Vorläuferzellen aus dem Körper des Säugetiers zu ermöglichen, und (b) das Protein nach Anspruch 1, 2 oder 3, das in der Matrix angeordnet und für diese Zellen zugänglich ist.

18. Vorrichtung nach Anspruch 16 oder Anspruch 17, wobei die Matrix eng gepacktes teilchenförmiges Material mit einer Teilchengröße im Bereich von 70 bis 850 µm (beispielsweise 70 bis 420 µm) umfasst.

19. Vorrichtung nach einem der Ansprüche 16 bis 18, wobei die Matrix Folgendes umfasst: (a) allogenen Knochen, beispielsweise demineralisierten, Protein-extrahierten, teilchenförmigen allogenen Knochen, (b) demineralisierten, Protein-extrahierten, teilchenförmigen, deglykosylierten xenogenen Knochen, (c) entmineralisierten, Protein-extrahierten, teilchenförmigen xenogenen Knochen, der mit HF oder einer Protease behandelt ist, (d) Materialien, die aus Kollagen, Hydroxyapatit, Calciumphosphaten (beispielsweise Tricalciumphosphat) und Polymeren ausgewählt sind, die Glycolsäure- und/oder Milchsäure-Monomere umfassen, (e) einen die Form behaltenden Feststoff aus locker haftendem teilchenförmigem Material, beispielsweise Kollagen, (f) einen porösen Feststoff oder (g) ein zerkleinertes Gewebe, beispielsweise Muskelgewebe.

20. Vorrichtung nach einem der Ansprüche 16 bis 19, die in der Markhöhle eines allogenen Knochens angeordnet ist.

21. Vorrichtung nach einem der Ansprüche 16 bis 20 zur Verwendung in der Therapie, beispielsweise zum Induzieren einer lokalen Knorpel- und/oder Ersatzknochen- oder heterotopen Knochenbildung in einem Säugetier durch Implantieren der Vorrichtung in ein Säugetier an einem Ort, der für migratorische Vorläuferzellen zugänglich ist.

22. Vorrichtung nach Anspruch 21 zur Bildung von geformten heterotopen Knochen, wobei die Form des heterotopen Knochens, der gebildet wird, derjenigen der implantierten Vorrichtung entspricht.

23. Verwendung des Proteins nach Anspruch 1, 2 oder 3 zur Herstellung des Folgenden: (a) einer Vorrichtung nach einem der Ansprüche 16 bis 21, oder (b) eines Medikamentes, wobei das Medikament oder die Vorrichtung zur Induktion einer lokalen Knorpel- und/oder Ersatzknochen- oder heterotopen Knochen-Bildung in einem Säugetier vorgesehen ist, indem das Medikament oder die Vorrichtung einem Säugetier an einem Ort eingebracht wird, der fü migratorische Vorläuferzellen zugänglich ist, zur periodontalen Behandlung, Knorpelinstandsetzung und zur Behandlung der Osteoarthritis und zur Korrektur von nichtverheilenden Frakturen, erworbenen oder angeborenen kraniofazialen oder anderen Skelettoder dentalen Anomalien.

24. Verwendung nach Anspruch 23 zur Bildung eines geformten heterotopen Knochens, wobei die Form des heterotopen Knochens, der gebildet wird, derjenigen der implantierten Vorrichtung entspricht.

25. Synthetisches Nukleinsäuremolekül zur Verwendung im Verfahren nach einem der Ansprüche 4 bis 8.

26. Wirtszelle zur Verwendung im Verfahren nach einem der Ansprüche 4 bis 8, die das synthetische Nukleinsäuremolekül nach Anspruch 25 enthält.

27. Wirtszelle nach Anspruch 26, die eine prokaryontische oder eukaryontische Wirtszelle ist, beispielsweise E. coli, Bacillus, Hefen oder Tierzellen (beispielsweise CHO oder Myeloma).

28. Isolierte DNA-Sequenz, die die Aminosäuresequenz des wie in einem der Ansprüche bis 3 oder 9 definierten Proteins kodiert.

Revendications

1. Protéine ostéogène comprenant une paire de chaînes polypeptidiques liées à l'état non réduit pour former une

espèce homodimère ou hétérodimère dont la conformation est telle que la paire de chaînes polypeptidiques est capable d'induire une ostéogenèse cartilagineuse lorsqu'elle est disposée à l'intérieur d'une matrice et implantée dans un mammifère, dans laquelle la protéine comprend une région contenant six résidus de cystéine disposés dans les positions relatives représentées dans la séquence :

LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXXQXXVX
XXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX,

séquence dans laquelle les lettres indiquent les résidus d'acides aminés d'un code standard à lettre unique et chaque X représente n'importe quel acide aminé, avec cette réserve que l'on exclut le dpp.

2. Protéine selon la revendication 1, dans laquelle la protéine comprend une région contenant sept résidus de cystéine disposés dans les positions relatives représentées dans la séquence :

CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXNHAXX
QXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXLXXYXXMXVXXCXCX.

3. Protéine selon la revendication 1 ou 2, à savoir une protéine recombinante que l'on obtient par expression dans une cellule hôte.

4. Procédé pour produire une protéine ostéogène synthétique active, comme défini à la revendication 1, ladite protéine comprenant une paire de chaînes polypeptidiques liées via des ponts disulfure pour produire une espèce dimère, le procédé comprenant les étapes consistant à :

(a) produire une séquence consensus d'acides aminés en comparant des séquences d'acides aminés de protéines de la famille génique TGF-bêta, ladite séquence consensus conservant le modèle de réticulation via des ponts disulfure présent dans la séquence :

(i) LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXN
HAXXQXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXL
XXYXXMXVXXCXCX;

ou

(ii) CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXX
XXXXNHAXXQXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXX
XXVXLXXYXXMXVXXCXCX;

séquence dans laquelle les lettres indiquent les résidus d'acides aminés d'un code standard à lettre unique et chaque X représente n'importe quel acide aminé ;

(b) construire un gène de synthèse encodant la séquence consensus d'acides aminés de l'étape (a) par exemple en assemblant deux oligonucléotides qui ont été synthétisés par voie chimique ;

(c) introduire le gène de synthèse obtenu par l'étape (b) dans une cellule hôte procaryote ou eucaryote ;

(d) exprimer le gène de synthèse pour produire une protéine ostéogène synthétique généralement admise ;

(e) analyser la protéine ostéogène synthétique généralement admise dans le but de déterminer le fait de savoir si elle est active en ce qui concerne l'induction d'une ostéogenèse cartilagineuse lors de son implantation dans un mammifère, et le cas échéant

(f) répéter les étapes (a) à (e) (ou procéder à une mutagenèse du gène de synthèse et répéter les étapes (c) à (e) lorsque la protéine ostéogène synthétique généralement admise se révèle inactive lors de l'analyse.

5. Procédé selon la revendication 4, dans lequel la séquence consensus d'acides nucléiques comprend la séquence :

(a) **LXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXN**
HAXXQXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXVXL
XXYXXMXVXXCXCX;

ou

(b) **CXXXXLXVXFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXX**
XXXXNHAXXQXXVXXXNXXXXPXXCCXPXXXXXXXXLXXXXX
XXVXLXXYXXMXVXXCXCX;

séquence dans laquelle les lettres indiquent les résidus d'acides aminés d'un code standard à lettre unique et chaque X représente n'importe quel acide aminé.

6. Procédé selon l'une quelconque des revendications 4 à 5, dans lequel le gène de synthèse de l'étape (b) comprend une séquence nucléotidique qui encode une chaîne polypeptidique synthétique qui possède le même nombre de résidus de cystéine dans les mêmes positions relatives que COP5, COP7, COP16 ou OP1.

7. Procédé selon l'une quelconque des revendications 4 à 6, dans lequel le gène de synthèse de l'étape (b) comprend une séquence nucléotidique qui encode une chaîne polypeptidique synthétique qui partage un ou plusieurs acides aminés à des positions correspondantes dans l'une quelconque des séquences : Vg1, DPP, OP1, CBMP-2a, CBMP-2b, CBMP-3,COP1, COP3, COP4, COP5, COP7 ou COP16.

8. Procédé selon l'une quelconque des revendications 4 à 7, dans lequel le gène de synthèse de l'étape (b) comprend une séquence nucléotidique qui encode une chaîne polypeptidique synthétique comprenant la séquence d'acides aminés :

```
        10        20        30        40        50
CKRHPLYVDFRDVGWNDWIVPPGYHAFYCHGECPFPLADHLNSTNHAIV
 RKRS K S S L QE VIS E FD Y  E A AY MPESMKAS   VI
   KE F E K I DN     L    N  S   Q ITK F P    TL
    Q    A  S        K
      60        70        80        90        100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
 SI HAI SEQV EP  A EQMNSLAI FFNDQDK I RK EE T DA H H
    RF    T   S    K DPV V  Y N S     H RN   RS
    N     S                  K         P    E
```

dans laquelle, dans chaque position dans laquelle on représente plus d'un acide aminé, l'un quelconque des acides aminés représentés peut se trouver dans cette position ; ou

```
     10        20        30        40        50
LYVDFRDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNSTNHAIV
 K S S L  QE VIS E FD Y  E A AY MPESMKAS   VI
 F E K I  DN     L    N  S   Q ITK F P    TL
     A    S      K
      60        70        80        90        100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKKYQDMVVEGCGCR
 SI HAI SEQV EP  A EQMNSLAI FFNDQDK I RK EE T DA H H
    RF    T   S    X DPV V  Y N S     H RN   RS
    N     S                  K         P    E
```

dans laquelle, dans chaque position dans laquelle on représente plus d'un acide aminé, l'un quelconque des acides aminés représentés peut se trouver dans cette position ; ou

(c)

```
1        10          20         30         40
CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD
                50         60          70
 HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS
     80         90        100
 VAMLYLNDQSTVVLKNYQEMTVVGCGCR;
```

ou

(d)

1 10 20 30 40
LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
50 60 70
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
80 90 100
ISVLYFDDSSNVILKKYRNMVVRACGCH;

ou

(e)

-5
HQRQA
1 10 20 30 40
CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
50 60 70
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
80 90 100
ISVLYFDDSSNVILKKYRNMVVRACGCH;

ou

(f)

1 10 20 30 40
LYVDFQRDVGWDDWIIAPVDFDAYYCSGACQFPSAD
50 60 70
HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
80 90 100
ISMLYLDENSTVVLKNYQEMTVVGCGCR;

ou

(g)

1 10 20 30 40

LYVDFQRDVGWDDWIVAPPGYQAFYCSGACQFPSAD

50 60 70

HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA

80 90 100

ISMLYLDENEKVVLKNYQEMVVEGCGCR;

ou

(h)

1 10 20 30 40

LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD

50 60 70

HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA

80 90 100

ISMLYLDENEKVVLKKYQEMVVEGCGCR.

9. Protéine selon l'une quelconque des revendications 1 à 3, dans laquelle la protéine comprend une chaîne polypeptidique synthétique contenant moins de 200 acides aminés.

10. Protéine selon l'une quelconque des revendications 4 à 8, dans laquelle la protéine comprend une chaîne polypeptidique synthétique contenant moins de 200 acides aminés.

11. Protéine selon la revendication 3, dans laquelle la cellule hôte est une cellule hôte procaryote ou eucaryote, par exemple des cellules de E. coli, de Bacillus, de levure ou des cellules animales (par exemple des cellules CHO ou des cellules de myélome).

12. Procédé selon l'une quelconque des revendications 4 à 8 ou 10 dans lequel la cellule hôte est une cellule hôte procaryote ou eucaryote par exemple des cellules de E. coli, de Bacillus, de levure ou des cellules animales (par exemple des cellules CHO ou des cellules de myélome).

13. Protéine selon l'une quelconque des revendications 1 à 3, dans laquelle la protéine est non glycosylée.

14. Procédé selon l'une quelconque des revendications 4 à 8 ou 10 dans lequel la protéine est non glycosylée.

15. Composition comprenant une protéine comprenant une paire de chaînes polypeptidiques liées via des ponts disulfure pour produire une espèce dimère, la protéine étant telle que définie à la revendication 1, 2 ou 3.

16. Dispositif ostéogène comprenant une matrice dans laquelle est disposée la protéine selon la revendication 1, 2 ou 3.

17. Dispositif ostéogène à des fins d'implantation dans un mammifère, le dispositif comprenant : (a) une matrice biocompatible, biodégradable in vivo définissant un support de dimension suffisante pour permettre la fixation, la

prolifération et la différenciation de cellules souches migratrices à partir du corps dudit mammifère, et (b) la protéine selon la revendication 1, 2 ou 3 disposée dans ladite matrice et accessible auxdites cellules.

18. Dispositif selon la revendication 16 ou 17, dans lequel ladite matrice comprend une matière particulaire fortement densifiée qui possède une granulométrie dans la plage de 70 à 850 µm (par exemple de 70 à 420 µm).

19. Dispositif selon l'une quelconque des revendications 16 à 18, dans lequel ladite matrice comprend : (a) de l'os allogène, par exemple de l'os allogène, particulaire, extrait d'une protéine, déminéralisé ; (b) de l'os xénogène déglycosylé, particulaire, extrait d'une protéine, déminéralisé ; (c) de l'os xénogène déglycosylé, particulaire, extrait d'une protéine, déminéralisé, qui a été traité avec du HF ou avec une protéase ; (d) de matière choisie parmi le groupe comprenant le collagène, l'hydroxy-apatite, des phosphates de calcium (par exemple le phosphate tricalcique) et des polymères comprenant des monomères d'acide glycolique et/ou d'acide lactique ; (e) un produit solide conservant sa forme, constituée d'une matière particulaire présentant une adhérence lâche, par exemple du collagène ; (f) un produit solide poreux ; ou (g) un tissu masticateur, par exemple un muscle.

20. Dispositif selon l'une quelconque des revendications 16 à 19, placé dans la cavité médullaire d'os allogène.

21. Dispositif selon l'une quelconque des revendications 16 à 20, à utiliser en thérapie, par exemple pour induire une formation locale de cartilage et/ou d'os enchondral ou hétérotope chez un mammifère par implantation du dispositif dans un mammifère à un site accessible à des cellules souches migratrices.

22. Dispositif selon la revendication 21, pour la formation d'os hétérotope configuré, dans lequel la configuration de l'os hétérotope à l'état formé épouse celle du dispositif implanté.

23. Utilisation de la protéine selon la revendication 1, 2 ou 3 pour la fabrication : (a) d'un dispositif selon l'une quelconque des revendications 16 à 21 ou (b) d'un médicament, le médicament ou le dispositif étant destiné à induire une formation locale de cartilage et/ou d'os enchondral ou hétérotope chez un mammifère en introduisant le médicament ou le dispositif dans un mammifère à un site accessible à des cellules souches migratrices pour un traitement parodontal, une réparation de cartilage et pour le traitement de l'arthrose ou encore pour corriger des fractures en l'absence de soudure, des anomalies craniofaciales acquises ou congénitales, et d'autres anomalies squelettiques ou dentaires.

24. Utilisation selon la revendication 23 pour la formation d'os hétérotope configuré, dans lequel la configuration de l'os hétérotope à l'état formé épouse celle du dispositif implanté.

25. Molécule synthétique d'acide nucléique à utiliser dans le procédé selon l'une quelconque des revendications 4 à 8.

26. Cellule hôte à utiliser dans le procédé selon l'une quelconque des revendications 4 à 8, qui contient la molécule synthétique d'acide nucléique de la revendication 25.

27. Cellule hôte selon la revendication 26, à savoir une cellule hôte procaryote ou eucaryote, par exemple des cellules de E. coli, de Bacillus, de levure ou des cellules animales (par exemple des cellules CHO ou des cellules de myélome).

28. Séquence d'ADN isolée qui encode la séquence d'acides aminés de la protéine telle que définie dans l'une quelconque des revendications 1 - 3 ou 9.

FIG. 1-1

COP1	COP3	COP4	COP5	COP7	VG1	DPP	OP1	CBMP2 a)	CBMP2 b)	CBMP3 (bov)	beta-Inhibin a)	beta-Inhibin b)	TGF-beta 1	TGF-beta 2	TGF-beta 3	IS	alpha Inhib	consensus choices
					C	C	C	C		C	C	C	C			C	C	c
					K	R	K	K	R	A	C	C	C			A	H	k,r
<P	P	P	P	P>	K	R	K	R		R	K	R	V	L	V	L	R	k,r
<N	N	N	N	N>	R	H	H	H		R	K	Q	R			R	V	h,k,r,q
<G	G	G	G	G>	H	S	E	P	S	Y	Q	Q	Q	P	P	E	A	p,s,e,q
L	L	L	L	L	L	L	L	L		L	F	F	L			L	L	l
Y	Y	Y	Y	Y	Y	Y	Y	Y		K	F	F	Y			S	N	y,f
V	V	V	V	V	V	V	V	V		V	V	I	I			V	I	v,i
D	D	D	D	D	E	D	S	D		D	S	D	D			D	S	d,e,s
F	F	F	F	F	F	F	F	F		F	F	F	F			L	F	f
Q	Q	S	S	S	K	S	R	S		A	K	R	R	K	R	R	Q	k,r,s
R	R												K	R	Q	A		
D	D	D	D	D	D	D	D	D		D	D		D			E	E	d
V	V	V	V	V	V	V	L	V		I	I	I	L			R	L	v,l,i
G	G	G	G	G	G	G	G	G		G	G	G	G			S	G	g
W	W	W	W	W	W	W	W	W		W	W	W	W				W	w
D	D	D	D	N	Q	D	Q	N		S	N	N	K				E	q,n,d,e,s
D	D	D	D	D	N	D	D	D		E	D	D	-				R	d,e,n
W	W	W	W	W	W	W	W	W		W	W	W	W				W	w
I	I	I	I	I	V	I	I	I		I	I	I	I	I	V	V	I	i,v
I	V	V	V	V	I	V	I	V		I	I	I	H			L	V	i,v
A	A	A	A	A	A	A	A	A		S	A	A	E			I	Y	a,s
P	P	P	P	P	P	P	P	P		P	P	P	P			P	P	p
V	P	P	P	P	Q	L	E	P		K	S	T	K			E	P	e,q,p*
D	G	G	G	G	G	G	G	G		S	G	G	G			T	S	g,s
F	Y	Y	Y	Y	Y	Y	Y	Y		F	Y	Y	Y			Y	F	y,f
D	Q	Q	Q	H	M	D	V	H		D	H	Y	H	N	Y	Q	I	h,d,n,q,y*
A	A	A	A	A	A	A	A	A		A	A	G	A			A	F	a

FIG. 1-2

```
Y F  F  F F :: N  Y  Y  F  :  Y  N  N   N      :  N  H  ::   y,f,n
Y Y  Y  Y Y :: Y  Y  Y  Y  :  Y  Y  Y   F      :  N  Y  ::   y,f,n
C C  C  C C :: C  C  C  C  :  C  C  C   C      :  C  C  ::   c
S S  S  H H :: Y  H  E  H  :  S  E  E   L A S: Q  H  ::   e,h,*
G G  G  G G :: G  G  G  G  :  G  G  G   G      :  G  G  ::   g
A A  A  E E :: E  K  E  E  :  A  E  S   P A P: V  G  ::   e,a*
C C  C  C C :: C  C  C  C  :  C  C  C   C      :  C  C  ::   c
Q Q  Q  P P :: P  P  A  P  :  Q  P  P   P      :  G  G  ::   p,a,g,q
F F  F  F F :: Y  F  F  F  :  F  S  A   Y      :  W  L  ::   f,y
P P  P  P P :: P  P  P  P  :  P  H  Y   I L L: P  H  ::   p
S S  S  L L :: L  L  L  L  :  M  I  L   W W R: Q  I  ::   l,m,i
A A  A  A A :: T  A  N  A  :  P  A  A   S      :  S  P  ::   p,a,s,t
D D  D  D D :: E  D  S  D  :  K  G  G          :  D  P  ::   d,e,s,g*
H H  H  H H :: I  H  Y  H  :  S  T  V   L      :  R  N  ::   h,r,n, s*
F F  F  F L :: L  F  M  L  :  L  S  P   L S A:     L  ::m,l,f,p,sa
N N  N  N N :: N  N  N  N  :  K  G  G   D      :  N  S  ::   n,k
S S  S  S S :: G  S  A  S  :  P  S  S          :  P  L  ::   s,a,p,g
T T  T  T T :: S  T  T  T  :  S  S  A   T      :  R  P  ::   t,s,a
N N  N  N N :: N  N  N  N  :  N  L  S   Q Q T: Y  V  ::   n
            ::             :     S  S          :  G  P  ::
            ::             :     F  F          :  N  G  ::
H H  H  H H :: H  H  H  H  :  H  H  H   Y H H: H  A  ::   h
            ::             :     S  T          :        ::
A A  A  A A :: A  A  A  A  :  A  T  A   S      :     P  ::   a,t,s
V V  V  V V :: I  V  I  I  :  T  V  V   K R T: V  P  ::   i,v,t
V V  V  V V :: L  V  V  V  :  I  I  V   V      :  V  T  ::   v,i,l
Q Q  Q  Q Q :: Q  Q  Q  Q  :  Q  N  N   L      :  L  P  ::   q,n
T T  T  T T :: T  T  T  T  :  S  H  Q   A S G: L  A  ::   t, s,a,g
L L  L  L L :: L  L  L  L  :  I  Y  Y   L      :  L  Q  ::   l,i
V V  V  V V :: V  V  V  V  :  V  R  R   Y      :  K  P  ::   v
N N  N  N N :: H  N  H  N  :  R  M  M   N      :  M  Y  ::   h,n r
```

FIG. 1-3

```
N N  N  S S :: S  N  F  S    : A  R  R   Q T T: Q  S  ::  n,s *
M M  M  V V :: I  N  I  V    : V  G  G   H I L: A  L  ::  i,v,l,
N N  N  N N :: E  N  N  N    : G  H  L   N    : R  L  ::  n
            ::               : V  S  N        : G     ::
            ::               : V              : A     ::
P P  P  S S :: P  P  P  S    : P  P  P   P    : A  P  ::  p,s,a
G G  G      :: E  G  E       : G  F  G   G E E: L  G  ::  g,e
            ::               :    A  T   A    : A     ::
K K  K  K K :: Q  K  T  K  S :    N  K   S    : R     ::  k,q,t,s
V V  V  I I :: I  V  V  I    : I  L  V   A    :       ::  i,v,l
P P  P  P P :: P  P  P  P    : P         A S S: P  A  ::  p,a,s
K K  K  K K :: L  K  K  K    : E  K  N        :    Q  ::  k
P P  P  A A :: P  A  A  A    : P  S  S   P    : P  P  ::  a,p,s
C C  C  C C :: C  C  C  C    : C  C  C   C    : C  C  ::  c
C C  C  C C :: C  C  C  C    : C  C  C   C    : C  C  ::  c
V V  V  V V :: V  V  A  V    : V  V  I   V    : V  A  ::  v,a,i
P P  P  P P :: P  P  P  P    : P  P  P   P S P: P  A  ::  p,s,a
T T  T  T T :: T  T  T  T    : E  T  T   Q    : T  L  ::  t,e,q
E E  E  E E :: K  Q  Q  E    : K  K  K   A D D: A  P  ::  q,e,d,k*
L L  L  L L :: M  L  L  L    : M  L  L   L    : Y  G  ::  l,m
S S  S  S S :: S  D  N  S    : S  R  S   E    : A  T  ::  s,n e,d
A A  A  A A :: P  S  A  A    : S  P  T   P    : G  M  ::  a,s,p,t
I I  I  I I :: I  V  I  I    : L  M  M   L    : K  R  ::  i,l,m,v
S S  S  S S :: S  A  S  S    : S  S  S   P T T: L  P  ::  s,a,p,t
M M  M  M M :: M  M  V  M    : I  M  M   I    : L  L  ::  v,m,i,l
L L  L  L L :: L  L  L  L    : L  L  L   V L L: I  H  ::  l,v,i
Y Y  Y  Y Y :: F  Y  Y  Y    : F  Y  Y   Y    : S  V  ::  y,f
L L  L  L L :: Y  L  F  L    : F  Y  F   Y    : L  R  ::  f,l,y
D D  D  D D :: D  N  D  D    : D  D  D   V I V: S  T  ::  d,n
E E  E  E E :: N  D  D  E    : E  D  D   G    : E  T  ::  d,e,
N N  N  N N :: N  Q  S  N  Y : N  G  E   R K R: E  S  ::  n,q,y*
E E  E  E E :: D  S  S  E  D : K  Q  Y   K T T: R  D  ::  e,d,s,t
```

FIG. 1-4

K	K	K	K	K	::	N	T	N	K		:	N	N	N	P			:	I	G	::	n,k
V	V	V	V	V	::	V	V	V	V		:	V	I	I	K			:	S	G	::	v,i
V	V	V	V	V	::	V	V	I	V		:	V	I	V	V	I	V	:	A	Y	::	v,i
L	L	L	L	L	::	L	L	L	L		:	L	K	K	E			:	H	S	::	l
K	K	K	K	K	::	R	K	K	K		:	K	K	R	Q			:	H	F	::	k,r
N	N	N	N	N	::	H	N	K	N		:	V	D	D	L			:	V	K	::	n,d,k*
Y	Y	Y	Y	Y	::	Y	Y	Y	Y		:	Y	I	V				:		Y	::	y
Q	Q	Q	Q	Q	::	E	Q	R	Q		:	P	Q	P	S			:	P	E	::	q,e,p,r
					::						:							:		TVP	::	
E	E	E	E	E	::	N	E	N	D	E	:	N	N	N	N			:	N	N	::	n,d,e
M	M	M	M	M	::	M	M	M	M		:	M	M	M	M			:	M	L	::	m
V	V	V	V	V	::	A	T	V	V		:	T	I	I	I	I	V	:	V	L	::	v,i,t,a
V	V	V	V	V	::	V	V	V	V		:	V	V	V	V			:	A	T	::	v
E	E	E	E	E	::	D	V	R	E		:	E	E	E	R	K	K	:	T	Q	::	e,d,r,k,
G	G	G	G	G	::	E	G	A	G		:	S	E	E	S			:	E	H	::	g,a,s,e
C	C	C	C	C	::	C	C	C	C		:	C	C	C	C			:	C	C	::	c
G	G	G	G	G	::	G	G	G	G		:	H	G	G	K			:	G	A	::	g,h
C	C	C	C	C	::	C	C	C	C		:	C	C	C	C			:	C	C	::	c
R	R	R	R	R	::	R	R	H	R		:	R	S	A	S			:	R	I	::	r,h,s,a,

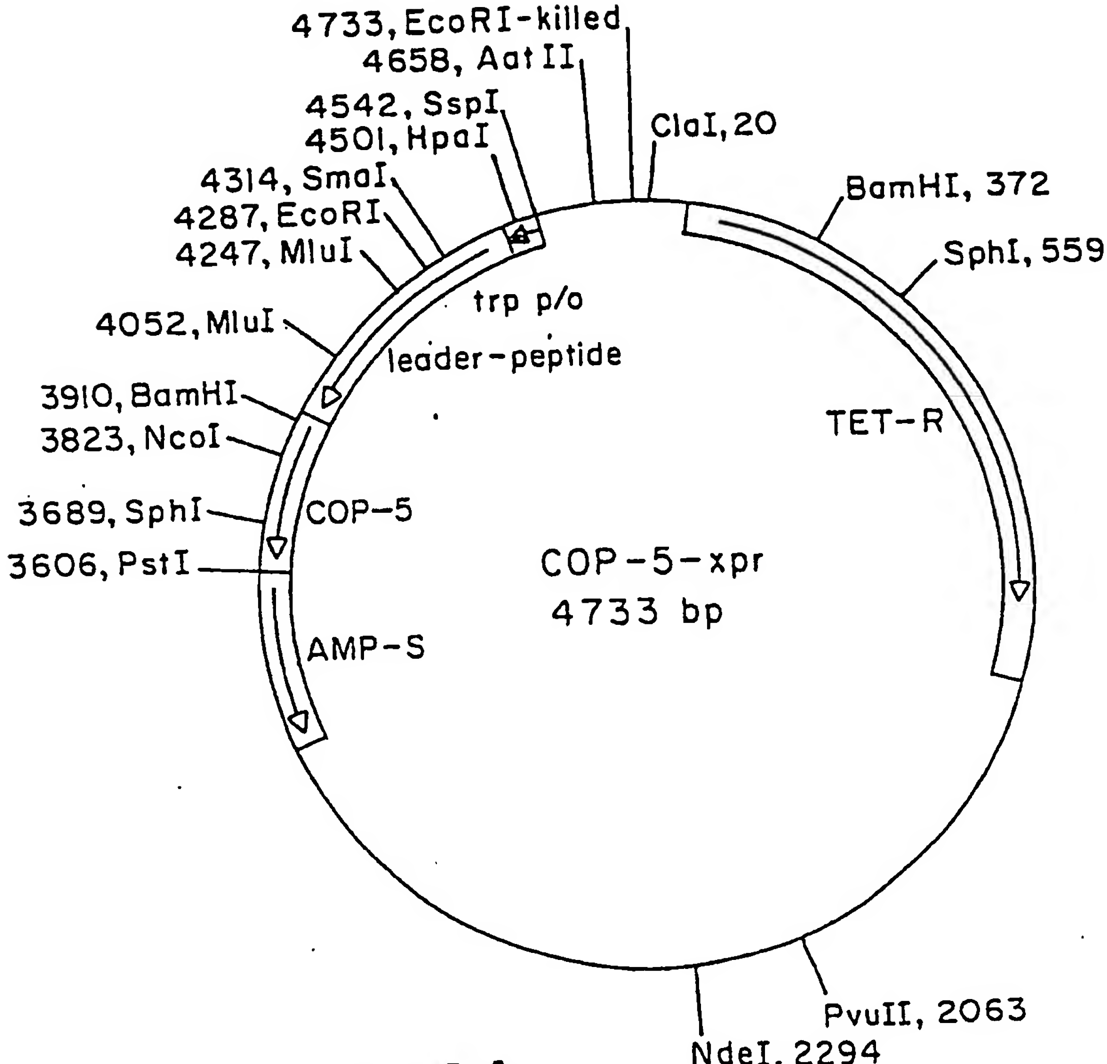
4733, EcoRI-killed
4658, AatII
4542, SspI
4501, HpaI
4314, SmaI
4287, EcoRI
4247, MluI
4052, MluI
3910, BamHI
3823, NcoI
3689, SphI
3606, PstI
ClaI, 20
BamHI, 372
SphI, 559
trp p/o
leader-peptide
TET-R
COP-5
AMP-S
COP-5-xpr
4733 bp
PvuII, 2063
NdeI, 2294

FIG. 2A

FIG. 2B-1

COP-5 fusion protein

```
         10        20        30        40        50
ATGAAAGCAATTTTCGTACTGAAAGGTTCACTGGACAGAGATCTGGACTC
 M  K  A  I  F  V  L  K  G  S  L  D  R  D  L  D  S
                                         BglII

         60        70        80        90       100
TCGTCTGGATCTGGACGTTCGTACCGACCACAAAGACCTGTCTGATCACC
  R  L  D  L  D  V  R  T  D  H  K  D  L  S  D  H

        110       120       130       140       150
TGGTTCTGGTCGACCTGGCTCGTAACGACCTGGCTCGTATCGTTACTCCC
L  V  L  V  D  L  A  R  N  D  L  A  R  I  V  T  P
        SalI                                    Sma

        160       170       180       190       200
GGGTCTCGTTACGTTGCGGATCTGGAATTCATGGCTGACAACAAATTCAA
 G  S  R  Y  V  A  D  L  E  F  M  A  D  N  K  F  N
I                        EcoRI

        210       220       230       240       250
CAAGGAACAGCAGAACGCGTTCTACGAGATCTTGCACCTGCCGAACCTGA
  K  E  Q  Q  N  A  F  Y  E  I  L  H  L  P  N  L
                 MluI       BglII     BspMI+

        260       270       280       290       300
ACGAAGAGCAGCGTAACGGCTTCATCCAAAGCTTGAAGGATGAGCCCTCT
N  E  E  Q  R  N  G  F  I  Q  S  L  K  D  E  P  S
                                HindIII

        310       320       330       340       350
CAGTCTGCGAATCTGCTAGCGGATGCCAAGAAACTGAACGATGCGCAGGC
 Q  S  A  N  L  L  A  D  A  K  K  L  N  D  A  Q  A
                 NheI                    FspI

        360       370       380       390       400
ACCGAAATCGGATCAGGGGCAATTCATGGCTGACAACAAATTCAACAAGG
  P  K  S  D  Q  G  Q  F  M  A  D  N  K  F  N  K

        410       420       430       440       450
AACAGCAGAACGCGTTCTACGAGATCTTGCACCTGCCGAACCTGAACGAA
E  Q  Q  N  A  F  Y  E  I  L  H  L  P  N  L  N  E
        MluI         BglII      BspMI+

        460       470       480       490       500
GAGCAGCGTAACGGCTTCATCCAAAGCTTGAAGGATGAGCCCTCTCAGTC
 E  Q  R  N  G  F  I  Q  S  L  K  D  E  P  S  Q  S
                    HindIII
```

FIG. 2B-2

```
        510       520       530       540       550
TGCGAATCTGCTAGCGGATGCCAAGAAACTGAACGATGCGCAGGCACCGA
 A  N  L  L  A  D  A  K  K  L  N  D  A  Q  A  P
      NheI                          FspI

        560       570       580       590       600
AGGATCCTAATGGGCTGTACGTCGACTTCAGCGACGTGGGCTGGGACGAC
K  D  P  N  G  L  Y  V  D  F  S  D  V  G  W  D  D
 BamHI               SalI

        610       620       630       640       650
TGGATTGTGGCCCCACCAGGCTACCAGGCCTTCTACTGCCATGGCGAATG
 W  I  V  A  P  P  G  Y  Q  A  F  Y  C  H  G  E  C
                          StuI       NcoI    BsmI+

        660       670       680       690       700
CCCTTTCCCGCTAGCGGATCACTTCAACAGCACCAACCACGCCGTGGTGC
 P  F  P  L  A  D  H  F  N  S  T  N  H  A  V  V
      NheI                           DraIII
                                    PflMI

        710       720       730       740       750
AGACCCTGGTGAACTCTGTCAACTCCAAGATCCCTAAGGCTTGCTGCGTG
Q  T  L  V  N  S  V  N  S  K  I  P  K  A  C  C  V
                               MstII

        760       770       780       790       800
CCCACCGAGCTGTCCGCCATCAGCATGCTGTACCTGGACGAGAATGAGAA
 P  T  E  L  S  A  I  S  M  L  Y  L  D  E  N  E  K
                       SphI

        810       820       830       840       850
GGTGGTGCTGAAGAACTACCAGGAGATGGTAGTAGAGGGCTGCGGCTGCC
 V  V  L  K  N  Y  Q  E  M  V  V  E  G  C  G  C
                    PflMI

        860
GCTAACTGCAG
R  *
    PstI
```

FIG. 3A

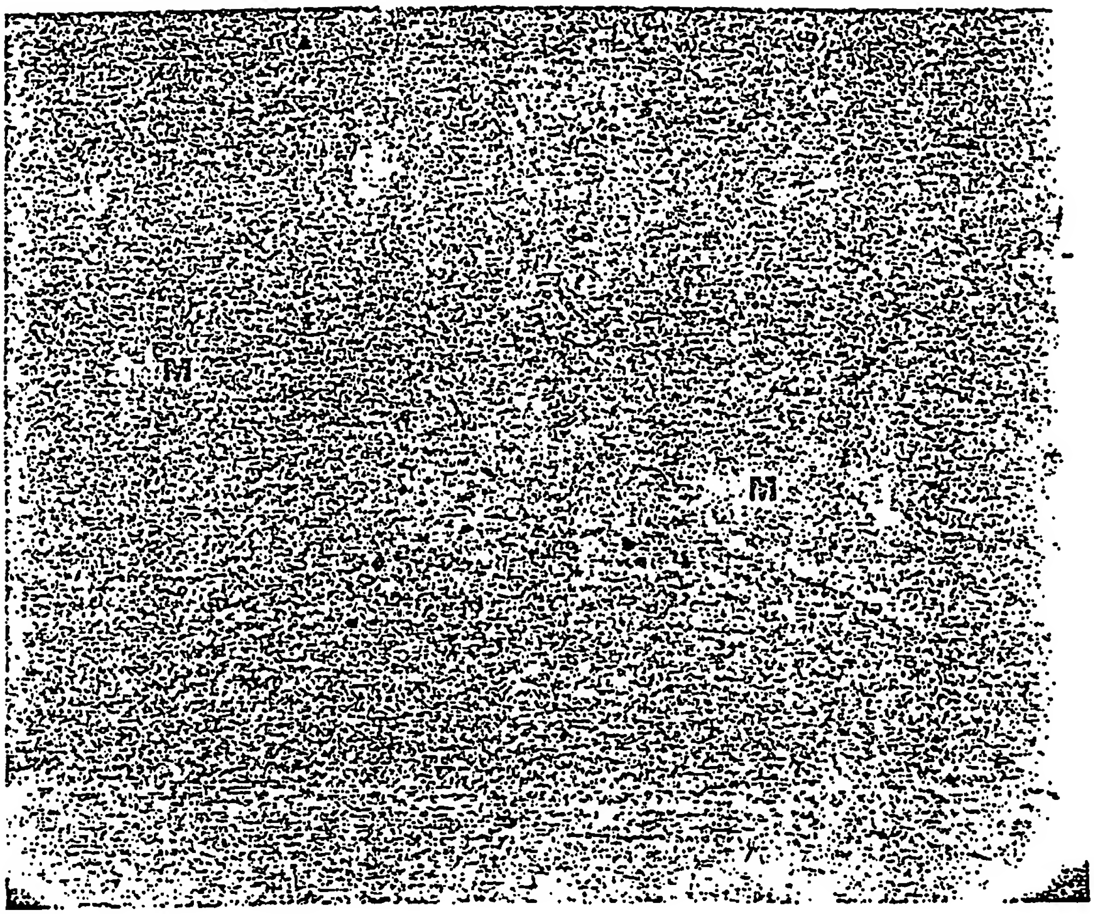

FIG. 3B

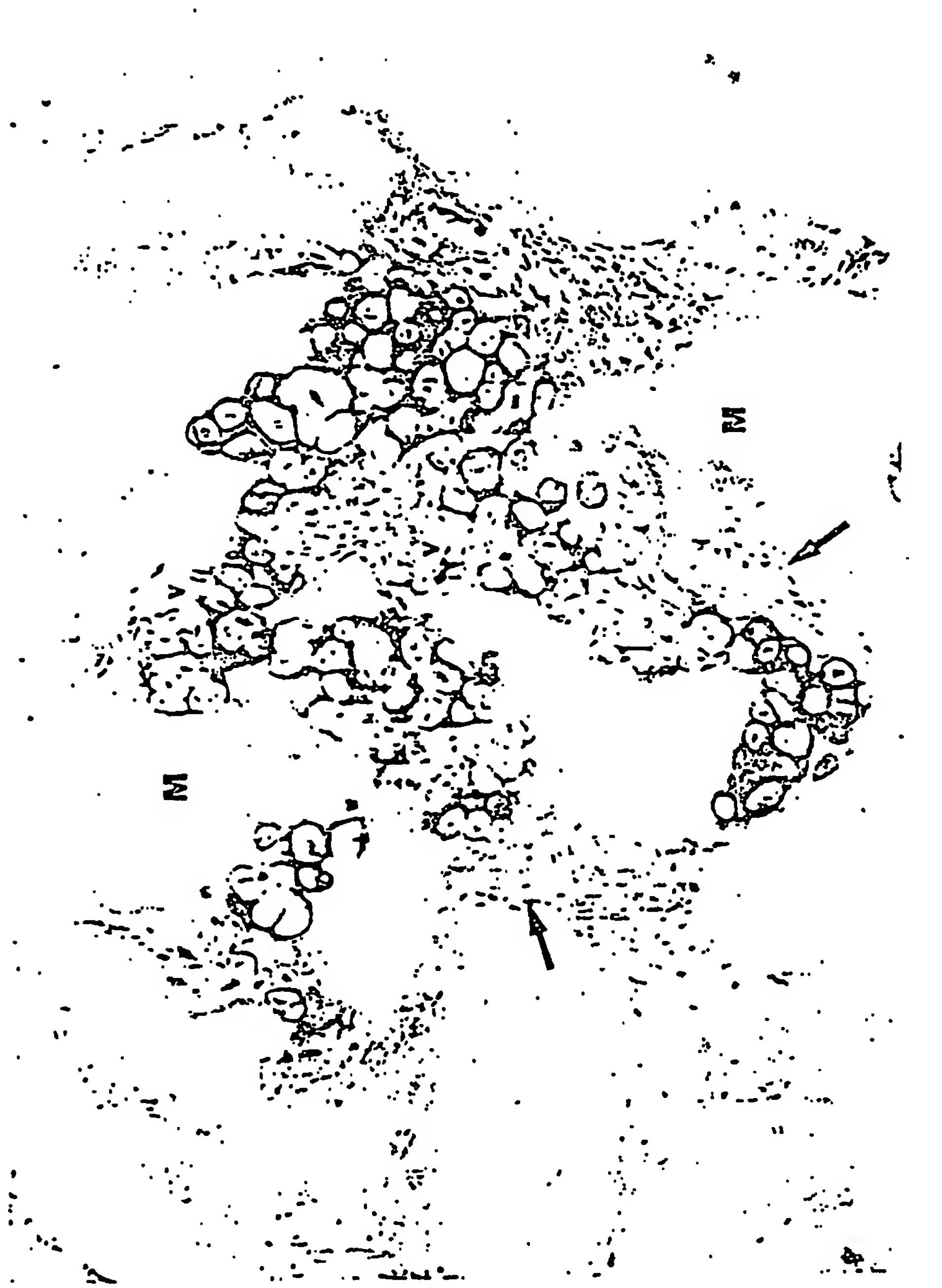

FIG. 4

```
         10        20        30        40        50
GATCCTAATGGGCTGTACGTGGACTTCCAGCGCGACGTGGGCTGGGACGA
 D  P  N  G  L  Y  V  D  F  Q  R  D  V  G  W  D  D

         60        70        80        90       100
CTGGATCATCGCCCCCGTCGACTTCGACGCCTACTACTGCTCCGGAGCCT
  W  I  I  A  P  V  D  F  D  A  Y  Y  C  S  G  A

        110       120       130       140       150
GCCAGTTCCCCTCTGCGGATCACTTCAACAGCACCAACCACGCCGTGGTG
C  Q  F  P  S  A  D  H  F  N  S  T  N  H  A  V  V

        160       170       180       190       200
CAGACCCTGGTGAACAACATGAACCCCGGCAAGGTACCCAAGCCCTGCTG
 Q  T  L  V  N  N  M  N  P  G  K  V  P  K  P  C  C

        210       220       230       240       250
CGTGCCCACCGAGCTGTCCGCCATCAGCATGCTGTACCTGGACGAGAATT
  V  P  T  E  L  S  A  I  S  M  L  Y  L  D  E  N

        260       270       280       290       300
CCACCGTGGTGCTGAAGAACTACCAGGAGATGACCGTGGTGGGCTGCGGC
S  T  V  V  L  K  N  Y  Q  E  M  T  V  V  G  C  G

        310
TGCCGCTAACTGCAG
 C  R  *
```

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